Addis Ababa University
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Chromosome study of bats from different localities in Ethiopia

By

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List of Abbreviations

APS$_4$ = Arba Minch Preparatory School specimen No. 4

AT$_3$ = Arba Minch Textile specimen No. 3

DD$_5$ = Dim-Dim Cave specimen No. 5

KT$_1$ = Koka Tannery Specimen No. 1

KT$_2$ = Koka Tannery Specimen No. 2

KT$_3$ = Koka Tannery Specimen No.3

ZA$_1$ = Ziway Antonio Residence Specimen No. 1

ZA$_2$ = Ziway Antonio Residence Specimen No. 2

ZA$_3$ = Ziway Antonio Residence Specimen No. 3

ZB$_2$ = Ziway Bridge Specimen No. 2

ZC$_1$ = Ziway Theology College Specimen No. 1

ZC$_2$ = Ziway Theology College Specimen No. 2

ZC$_3$ = Ziway Theology College Specimen No. 3

ZF$_1$ = Ziway Fruit Corporation Specimen No. 1

ZF$_2$ = Ziway Fruit Corporation Specimen No. 2

ZF$_3$ = Ziway Fruit Corporation Specimen No. 3

ZK$_1$ = Ziway Kobo Specimen No. 1

ZK$_2$ = Ziway Kobo Specimen No. 2

ZKM = Ziway Kobo Muz

ABSTRACT

Bats were captured by using nets and hand picking and then chromosome preparations were made from bone marrow cells according to Lee and Elder's (1980) method cited in Hillis et al. (1996) with some modifications. Somatic metaphase chromosomes of bat specimens collected from Arbaminch, Koka, Merehabete and Ziway regions in Ethiopia were studied. The specimens were identified belong to the following families and species. *Pipistrellus pipistrellus* and *Scotophilus dinganii or S. viridis* (family Vespertilionidae), *Micropteropus pusillus* (family Pteropidae), *Chaerephone pumila* (family Molossidae) and a taxonomically yet unidentified specimen collected from Merehabete region. Five karyotypes, differing in chromosome number, fundamental number and chromosome morphology were identified. Accordingly, the karyotype of *Micropteropus pusillus* consists of 2n=35, FN=70 and in rare cases 2n=36, FN =72 with all the chromosomes being biarmed; *Pipistrellus pipistrellus* 2n=36, FN=52 with metacentric and acrocentric chromosomes; *Scotophilus dinganii(S.viridis)* 2n=36, FN=54 with metacentric/ acrocentric chromosomes; *Chaerephon pumila* 2n=48, FN=60 with metacentric, acrocentric and telocentric chromosomes;Merehabete specimen 2n=42,FN=82 with 40 biarmed and two telocentric chromosomes. The present karyotypes differed from previous reports either in 2n number, or fundamental numbers or chromosome morphology.

Key words or phrases: Bat, 2n number, Fundamental number, Karyotype, Ethiopia, Chromosome.

(Emballonuridae and Molossidae, aptly called sheath – tailed and free-tailed bats) have the tail moving freely in a pocket of skin (Yalden and Morris, 1975).

Most bats are only active at night, but island species in the absence of birds of prey, are often also active by day, and a few bats of most species will occasionally fly during daytime (Macdonald, 1984). Flying, especially at night, poses problems of obstacle avoidance and navigation, but facilitates food finding which may be patchily distributed in space and time. Although some bats, such as Old World flying foxes, have excellent sight, most bats rely upon highly acute hearing, which, with often-complex sound production, enables bats to navigate, feed and locate roosts by echolocation. Many bats, particularly the fruit eating species, have a keen sense of smell (Macdonald, 1984).

Bats, are nocturnal animals, roosting in caves; holes or crevices; and the open, usually hide away during the daytime where they may be secured from enemies and disturbance. Within the roosts, bats with few exceptions, suspend themselves by the claws of the hind feet, head downwards, the soles of both feet almost invariably facing the same way the ventral direction (Rosevear, 1965).

Bats, because of their small size and mobility, are variously susceptible to disturbance. The expansion and intensification of human land use contributed to the loss, destruction and increased isolation of habitat patches (Terborgh, 1974). Although little is known about the specific characteristics of roost sites, the loss of large trees and snags (i.e. dead trees due to fire and firewood collection) is expected to decrease roosting sites of bats and increase distances to foraging areas (Fenton *et al.,* 1998). This movement between roosting and foraging sites may demand high energetic costs. The reproductive success of females may also be affected due to long foraging distances as energy demand increases (Barclay, 1989).

The mammals of Ethiopia, in common with most other aspects of the fauna, are relatively poorly known. Little has been published on the subject in recent years, older reports are widely scattered throughout the zoological literature and the conclusions of the past workers have often been limited by a lack of comparative material. This is particularly true for bats where most of the literature is found in the form of checklists and museum voucher specimens (Largen *et al*., 1974).

Despite their diversity, widespread occurrence and, in some cases, numerical abundance, the flying ability of bats, their nocturnal habits and wary of human contact have contrived to keep them out of the way of zoologists until fairly recently. The classification of bats suffers primarily from the lack of adequate data. Many cryptic or sibling species are distinguishable on the basis of their karyotypes, they may be morphologically indistinguishable but biologically distinct (Fredga, 1977). Chromosomes can be studied as a morphological manifestation of the genome in terms of their microscopically visible size, shape, number and behaviors during meiosis and mitosis. Karyological approaches to systematics and taxonomy make use of the wealth of characters that chromosome sets provide and the detailed information on homology relationships between karyotypes. Karyological analysis provided important information for the evaluation of the systematic position and phylogenetic diversification of bats (Harada, 1988). The primary objective of this study was to provide information about the diversity of Ethiopian bats at chromosomal level. This will also contribute some new data important for taxonomic and evolutionary study of Ethiopian bats.

2. LITERATURE REVIEW

2.1 Bat Taxonomy

For a number of reasons, the study of the systematic of bats has not progressed as rapidly as that of some other mammalian groups. The major forces in chiropteran evolution have been those associated with flight and feeding (Findley and Wilson, 1982 in Owen, 1988), and bat taxonomists have not been in agreement on how best to evaluate these morphologic characters in systematic analyses.

The classification of bats suffers primarily from the lack of adequate data. New species are constantly being discovered and described as collecting techniques improve. Existing taxonomy is based mainly on external form (morphology), but results of cytological and modern biochemical techniques are beginning to demand reassessment of bat taxonomy. Ecological research is also providing clues to separate sibling species that were previously overlooked. As information improves some regrouping is necessary (Macdonald,1984).

The order chiroptera shows diversity both in form and mode of living. Nearly one quarter of mammalian species are bats. They form the largest order of mammals after the rodentia. Seventeen families of bats are known which consist of about 175 genera and about 800 species (Kingdon, 1974). However, these numbers fluctuate from time to time and from author to author due to new findings of species as well as merging of some species or genera together by different authors (Jepsen, 1966 in Wimsatt, 1970). The order chiroptera is divided into two suborders: megachiroptera and microchiroptera. The family Pteropodidae constitutes the whole of the suborder megachiroptera or fruit eating bats. All the other families are classified as suborder microchiroptera or insectivorous bats.

Insect eating bats form a diverse group; with about 756 species divided over 16 families worldwide and constitute 70% of the entire order (Wilson *et al.,* 1996). In East Africa, 69 species of insect eating bats are recognized (Kingdon, 1997). Insect eating bats have clawless wing, complex ears, and generally but not exclusively smaller than the fruit eating bats.

The family Pteropodiadae contains 166 species (Wilson *et al.,* 1996) exclusively confined in the tropics and subtropics (Nowak, 1991). In East Africa, 18 species of fruit eating bats have been recorded (Kingdon, 1997). The fruit eating bats are very readily distinguishable from insect eating bats by the presence, in all but a few East Indian and Pacific genera, of a claw on the index finger as well as on the thumb. Fruit eating bats have a very distinctive head in relation to the body and have large, endearing eyes that enable a powerful vision in place of echolocation in insect eating bats. Fruit bats have funnel shaped ears.

According to Largen *et al.* (1974) nine families consisting of 31 genera and 74 species of bats have been recorded in Ethiopia (Table 1). Twenty-three species of bats collected from different localities of Ethiopia have been deposited in the Natural History Museum at Science Faculty of Addis Ababa University (personal observation).

Table 1. Ethiopian bat* families and genera with number of species according to Largen *et al. (1974)*.

No.	Family	Genus	Number of Species
1	Pteropidae	*Hypsignathus*	1
		Epomoghorus	4
		Micropterapus	1
		Eidolon	1
		Rousettus	3
2	Rhinopomatidae	*Rhinopoma*	2
3	Emballonuridae	*Taphozous*	3
		Coleura	1
4	Nycteridae	*Nycteris*	3
5	Megadermatidae	*Lavia*	1
		Cardioderma	1
6	Rhinolophidae	*Rhinolophus*	8
7	Hipposideridae	*Hipposideros*	5
		Triaenops	1
		Asellia	2
8	Vespertilionidae	*Eptesicus*	3
		Pipistrellus	4
		Mimetillus	1
		Glauconycteris	1
		Laephotis	1
		Plecotus	1
		Barbasteila	1
		Miniopterus	1
		Mintopterus	1
		Nycticeius	3
		Myotis	4
		Scotophilus	2
		Kerivoula	2
9	Molossidae	*Otomops*	1
		Platymops	1
		Tadarida	10

*** Note:** Largen *et al*. (1974) constitute Eritrea as part of Ethiopia

2.2 Genealogy of Bats

Fruit bats and insect bats share a similar pattern of bare skin webbing between elongated fingers. Both groups have also lost most of the nails on the toes and these have evolved into sharp hooks. In spite of the marked similarities, the two groups exhibit numerous less obvious differences, which suggest that insect bats share a very ancient common ancestry with insectivores while the fruit bats may have more recent affinities with primates. Pending confirmation of separate origins (which would require two orders) fruit bats and insect bats remain distinct suborders (Kingdon, 1997).

In the absence of fossil “missing links”, one can only speculate, using what is known about bat morphology and habitats. A vital clue is provided by the basic similarity between the cheek teeth of bats and those of shrews and moles belonging to the order insectivora, instead of the blunt cusps found on primate molars. For example, the teeth of these animals have sharp pointed crests, which serve to chop up the tough exoskeletons of their invertebrate prey. It is reasonable to infer that bats may be derived from the more ancient insectivores, and certain similarities of anatomical detail, for example, the microstructure of their tooth enamel, supports this view. Both bats and insectivores are mainly nocturnal. Both bats and insectivores produce ultrasound but insect bats have used it to develop complex forms of echolocation (Yalden and Morris, 1975).

2.3 Geographic Distribution

Fruit bats probably evolved in South East Asia where the greatest variety and range of forms still exist. In Africa, there is a clear division between rainforest species (living in equatorial and mainly western regions) and Savannah species preponderant in East Africa. Rousette bats, a mainly Asiatic group, span both regions, and the flying foxes, another Asiatic group, occur only on Indian Ocean islands off Africa (Kingdon, 1997).

Insect bats have a worldwide distribution and many species in the temperate region (especially vesper bats) are able to hibernate, thus benefiting from the rich seasonal insect fauna of cool temperate regions. A few bat species of temperate region migrate (but mostly within continental boundaries). Several groups have close relatives, which occur in Africa, South Asia and Australia. One of these is a migrant and others, such as free tailed bats, are also strong, long–distance fliers. Some bats, such as the strictly forest dwelling horseshoe and leaf-nosed species appear to have diverged many millions of years ago but they occupy rather stable ecological niches and therefore resemble each other because they have remained very conservative (Kingdon, 1997). The range of forms and global distribution of bats is greater than that of any other order of mammals, a diversity that relates both to the mobility and the great age of this group.

In the higher latitudes of both the northern and southern hemispheres, winter means a shortage of food, especially for insectivorous animals like many bat species. In response to this, most insectivorous bats in these regions hibernate, though some may migrate to warmer climates. The ability of tropical insectivorous bats to enter periodic torpidity is a useful preadaptation for the invasion of temperate regions, and may well have been the physiological base from which long term hibernation evolved (Yalden and Morris, 1975).

Bats have been recorded from about an altitude of 7000 feet in West Africa, on the Cameroon Mountain; at 13000 feet in the East African highlands and at considerably greater heights in other continents (Rosevear, 1965). In Ethiopia, the range of altitude from where bats were recorded is from sea level to 3300m at Danka river, near Dinshu (Largen *et al.,* 1974). Bats usually inhabit forest areas and savanna grasslands with nearby water supply like streams, rivers, etc. (Bates and Harrison, 1997). Table 2 illustrates this.

Table 2. Sites in Ethiopia that are rich in bat diversity with their geographic coordinates, altitude and number of species according to Largen *et.al.* (1974).

No.	**Collecting Site**	**Geographic Coordinates**	**Altitude**	**Number of Species**
1	Awash National park	08^{0}54’N 39^{0}55’E	1000m	16
2	Bahadu	10^{0}06’N 40^{0}36’E	600m	8
3	Sof Omar	06^{0}54’N 40^{0}48’E	1340m	11
4	Koka	08^{0}26’N 39^{0}01’E	1700m	9
5	Lake Abiata	07^{0}37’N 38^{0}39’E	1590m	13
6	Arba Minch	05^{0}57’N 37^{0}32’E	1400m	11
7	Bulcha Forest	06^{0}11’N 38^{0}10’E	1800m	9
8	Didessa River	09^{0}02’N 36^{0}09’E	1200m	15
9	Gambela	08^{0}15’N 34^{0}35’E	515m	27

2.4 Flight and Feeding

The variety of body forms, which is the basis of current classification of bats, reflects the adaptations each species has made in response to the ecological niche in which it has evolved and helped to differentiate. The wing pattern is essentially similar in all bat species, but differences in shape reflect the variety of ecological niches and feeding behavior exhibited by bats (Macdonald, 1984).The third digit is the longest and extends to the wing tip; the ratio of its length relative to the fifth digit, which is a measure of the wing width, characterizes the flight pattern. Bats with the third digit about 1⅓ times longer than the fifth have short, broad wings, low aspect ratios and are generally slow flyers. The wings are highly maneuverable. Aspect ratios are the ratio of wing span to average wing width. Bats with the third digit about twice as long as the fifth have long thin wings and high aspect ratios and fly rapidly. The wings, are not very maneuverable (Macdonald, 1984).

As the name suggests, the fruit eating bats are almost exclusively frugivorous. However, they feed on other diets such as pollen and nectar, some of the fleshier parts of flowers or even the whole of large and succulent buds. They detect the fruits using their well-developed sense of smell. Fruit bats have large brains, large tongues, extrusible lips, a wide maw and flexible cheek pouches (Kingdon, 1997).

Insects are the mainstay diet of microchiropterans although they are known to feed also on other preys, including insects, caught in flight and at rest; other arthropods, including scorpions, woodlice and shrimps; vertebrates, including mice, other bats, lizards, amphibians and fish, and blood of mammals or birds. Insect bats have complex teeth to chop up the tough exoskeletons of their invertebrate prey. Some microchiroptera, however, feed on plant materials, including pollen, nectar and particularly fruit. Fruit or blood-eating microchiropteran bats appear to use vision and olfactory in addition to echolocation to find prey (Eisenberg and Wilson, 1978). While most bats specialize on a relatively narrow diet range, with none more limited than the common vampire, which feeds throughout its life on the blood of mostly one breed of cattle, some like the greater spear nosed bat, are omnivorous, feeding on vertebrates, insects and fruit (Kingdon, 1997).

2.5. Reproduction

In some families there is nothing beyond the actual sexual organs themselves to distinguish between male and females; in others certain special features are characteristic of adults of one sex or the other, usually the males (Rosevear, 1965). In megachiroptera adult females have ‘false-nipples’ as well as milk-bearing teats and these form the firmest point of attachment for the infant (Kingdon, 1997). In so far as secondary sexual modifications of body form are concerned, *Hypsignathus monstrosus* is unique among fruit bats in the tremendous outgrowth of the lips in the males into great fleshy pads (Rosevear, 1965).

The males in some genera of bats are appreciably larger than the females, but the most striking secondary sexual characters are the existence in the males of the genera *Epomophorus, Epomops, Micropteropus and Nanonycteris* of large white plumes, "shoulder tufts" or "epaulets", which may be concealed within a pouch or turned outwards and visibly displayed at will. In *Rousettus angolensis* and *Megaloglossus woermanni* the adult males carry a collar of stout hairs, arising from glandular areas of the skin.

Many species exhibit sexual dimorphism, which often involves glandular areas in the male sometimes associated with differentiated hairs, tracts, tufts, sacs or pouches. According to Kingdon (1974) some sites for glandular areas are listed below:

Genera/Family	**Glandular areas**
Epomophorus	Shoulder pocket
Taphozous	Gular pouch
Rhinolophus landeri	Axillary tuft in "armpit"
Hipposideros Commersoni	Forehead pocket
Vespertilionids	Muzzle and other facial glands
Tadarida cistura	Anal sacs

Most species of microchiroptera have pungent glands, which fluctuate in size and appear to play a role in reproductive behavior in most instances (Kingdon, 1997). Colour is another general character, which seems to vary with sex in certain specific cases, the males being of a livelier hue, as in *Eidolon*. In general, secondary characters are less marked in microchiroptera (Rosevear, 1965).

The reproduction cycles of bats often include interesting, even unique, features, especially where modifications have arisen because the breeding cycle is interrupted by some other major event such as hibernation. Delayed fertilization (sperm storage) is a variant on the normal patterns of mammalian reproduction, which is unique to bats. Another peculiar variant on the standard mammalian reproductive cycle is that of delayed development. Functionally, delayed development serves to extend the gestation period so that the young are born at the best time of the year (Orr, 1970).

The gestation period varies from 50 to 60 days in small vespertilionid bats of the genera *Pipistrellus* and *Myotis* to as much as 8 months in the vampire (*Desmodus rotundus*) (Orr, 1970). In general, within a related group larger size appears to be correlated with a longer period of intrauterine life.

Normally, most bats produce one offspring per litter and one litter per year, but a number have twins and the red bat in North America averages three. In northern Europe, Pipistrelles produce a single offspring, but in more southerly areas twins are common. Since among recorded births twins are more frequent in well fed captive bats than in the same species in the wild, twinning is probably related to better nutrition (Kingdon, 1997).

Most microchiroptera are born pink and naked, but the skin becomes pigmented quite soon. Usually, the eyes are closed at birth. The young megachiroptera are often well furred and have their eyes open. At birth the megachiroptera is in a more advanced stage of development than the majority of microchiroptera. Thereafter, although the former at first continue to develop quickly, their pace becomes slower and they are eventually overtaken by the microchiroptera, which abandon the mother and fly at an earlier age (Rosevear, 1965). Reliable estimates of postnatal growth rates and age in bats are essential for characterizing the physiological, ecological, and behavioral changes that occur during the postnatal period (Hoying and Kunz, 1998). Maximum longevity for the little brown bat and probably many others is in excess of 30 years, but very few bats in any population will achieve that age (Kingdon, 1997). Average life span is often about 4 -5 years.

Cryan *et al.* (2000) found a significant inverse relationship between elevation and relative abundance of reproductive females. Relative abundance of reproductive females decreased as elevation increased. Reproductive females may be constrained from roosting and foraging in high elevation habitats that impose thermoregulatory costs and decrease foraging efficiency. Failure to account for sex differences in distributional patterns along elevation gradients may significantly bias estimates of population size.

Reproductive females that move from cold hibernacula to warm maternity sites could avoid costs associated with long range migrations by making shorter movements among elevations, assuming some universal effect of latitude and elevation on temperature. Temperature is a significant factor influencing roost selection and distribution of many temperate region bat species (Kunz, 1982). Thermal characters of roosts influence energy expenditure of bats (Kurta, 1986). Although cold induced torpor can ameliorate energy demands in reproductive females, it can delay fetal development (Hoying and Kunz, 1998). Such delays lengthen gestation, thus lowering female reproductive rates and increasing probability of early winter mortality of young (Grindal *et al.,* 1992).

2.6 Importance of Bats

The sinister association of bats with evil goes hand in hand with their ancient employment in human affairs: as essential ingredients of sorcery and magic, where their contribution is usually seen as wholly malevolent. Paradoxically, in the allied fields of witchcraft and pseudomedicine they are often considered beneficial, their blood, hearts and hair being regarded as particularly potent for marking both medicine and mischief. Various bat preparations were thought to give strength and cure rheumatism. Today bats are still used in medicine, though happily not as a pharmaceutical ingredient; their modern role is as experimental tools (Yalden and Morris, 1975).

Bats are exterminated in large numbers because they are alleged to spread rabies while others “suck” human blood. This is far from the truth because no record of the spread of rabies by bats has been recorded in Africa and vampire bats, which “suck” human blood, do not occur in Africa. In all of Asia, Europe, Australia and the Pacific Islands, only two people are suspected of dying from bat – transmitted rabies per year (Miller, 1988). Bats are also exterminated indiscriminately for bad odour in buildings where they roost. Yet bats are very important with a role to play in ecology and evolution. For instance, insect eating bats are the major predators of harmful insects and help to control insect populations, including mosquitoes that transmit malaria and those that damage human crops. The fruit eating bats predilection for mangoes, figs, guavas, bananas and other commercially valuable fruit types may result in regarding bats as pests, but they mainly eat wild fruits and berries in the forests. Contrary to the above fears associated with bats, some of their beneficial aspects are described below.

1. Bats are used in medicine as experimental tools. The transparency of the bat's wing permits a microscopic study of wound healing in a living animal (Yalden and Morris, 1975). The same property reveals details of blood circulation in the vessels of the wing; movements of blood cells and dyes are clearly visible and the rates at which drugs are eliminated from the body can be measured.
2. Understanding the control and effects of hypothermia is important, since many major surgical procedures are now carried out on humans at reduced body temperatures, when the body temperature is less susceptible to damage and requires less oxygen. Research on the physiological mechanisms of hibernation in bats and other mammals is relevant with this regard.
3. Hibernating bats can in fact survive periods of 200 days or so under experimental conditions. *Eptesicus fuscus* have been kept hibernating in refrigerators for as long as 344 days before dying of starvation, far longer than would be needed to survive a winter (Yalden and Morris, 1975). Such long survival periods are not possible in the wild simply because constant conditions are not available. Investigators have also shown that during hibernation animals may age more slowly and they are resistant to ionizing radiation. Both factors could become important in deferring human senility and might even be essential if space travel, including journeys lasting many years is ever to become a feasible proposition.
4. Bats that form large colonies, especially in the tropics, provide valuable sources of high quality meat, and their guano (excrement) is used and traded as fertilizer.
5. Many of the frugivorous bats, despite their depredations, may actually promote the development and dispersal of the species on which they feed. Some of the nectivorous bats also have an important role in the cross-pollination of their food plants. Twenty or more genera, mostly trees and shrubs, depend wholly or mainly upon bats for the pollination of their flowers (Yalden and Morris, 1975). For instance the decline of some flying foxes populations is having serious effects on crops, and tropical forest and savanna habitats because of their importance for pollination and seed dispersal,
6. By virtue of their feeding habits, bats often play a key role in the ecology of certain major habitats, aiding in the dispersal or suppression of various species and promoting the energy flow of certain ecosystems. The feeding activities of bats are

often the basis of the whole food web of the cave ecosystem. In caves, mines and similar underground habitats all the food necessary to maintain the resident cave animals must be imported from elsewhere: a task performed by bats.

2.7 Chromosome Features and Importance in Systematics

Chromosomes are almost entirely composed of DNA and proteins, and in all eukaryotes, the general structure and chemical composition of the chromosomes are in good agreement. However, the amount of DNA varies, and also the way in which the DNA is packed: the size, number and shape of the chromosomes varies considerably.

Chromosome studies can contribute an array of information independent from morphological, biochemical, behavioral and other characters that are used for phylogenetic analysis. Chromosomes can be studied as a morphological manifestation of the genome in terms of their microscopically visible size, shape, number, and behavior during meiosis and mitosis.

2.7.1. Mechanisms of Variation in Chromosome Number

The number and morphology of chromosome is characteristic of species. In general, a high chromosome number favors genetic recombination; a low number favors conservation of established linkage groups (Fredga, 1977). Few and large chromosomes may run into trouble at mitoses or meiosis due to limitation in length of the metaphase spindle. A high number of small chromosomes may increase the risk for non – disjunctional events with aneuploid cells or unbalanced gametes as a result (Fredga, 1977). Compared with amphibians, reptiles and birds, mammals have a wide range of variation in chromosome numbers (Fredga, 1977).

Most organisms are diploid, or have two sets of chromosomes. In addition to the numbers, the chromosome complement of a species may be characterized by the total number of chromosome arms, which is referred to as nombre fundamentale (NF) or fundamental number. According to Robbins and Baker (1978), the fundamental number (number of chromosome arms) has two definitions. The fundamental number includes all the arms of the sex and autosomal chromosomes. In the other definition, it includes only the arms of the autosomal chromosomes and is referred to as autosomal fundamental number (NFa).

Chromosomal rearrangements may alter the number of chromosomes, the number of chromosome arms, or both. Some kinds of rearrangements produce obvious chromosomal changes, while other kinds may be less obvious. As a result of chromosomal rearrangements, genes may be duplicated or deleted, or their sequence or linkage group may be changed. Change in the position of a gene may affect its action, a phenomenon known as position effect (Wahl *et. al,* 1984).

A Robertsonian rearrangement is the result either of the fusion of two centromeres into one, or the fission of one centromere into two, resulting in the formation of a single chromosome from two acrocentric or telocentric chromosomes or forming two telocentric chromosomes from one chromosome, respectively. This kind of rearrangement changes the chromosome number but not the arm number. Although Robertsonian fusion is one of the more common types of chromosomal rearrangement (Fredga, 1977), Robertsonian fission appears to be relatively rare in mammals. The situation in which a species shows a large variation of chromosome numbers due to Robertsonian rearrangements is called a Robertsonian fan.

Odd numbers of chromosomes are found in some individuals (Gropp and Winking, 1972). This represent cases where one member of each of two pairs of acrocentrics have fused to form a metacentric, but their respective homologs have remained separate. If one of the sex chromosomes is involved, the result will be that males and females of a species will have different numbers of chromosomes.

A tandem fusion is a fusion of two chromosomes in which the end of one chromosome is fused either to the end or to the centromere of another chromosome. Tandem fusion and karyotypic fissioning (fissioning of the entire complement of chromosomes) change both chromosome number and arm number. The most interesting example of this kind of variation is the case of the muntjacs. One species, *Muntiacus muntjac*, has only six chromosomes in the female, and seven in the male (Wurster and Benirschke, 1970). Another species, *M.reevesi,* has 46 chromosomes in both male and female (Liming *et. al.,* 1980). Comparison of banding patterns suggests that essentially the same genetic material is present in both species, since there is a one – to – one correspondence of bands, and indeed they appear very similar. However, in order for the chromosome number to be so drastically different, it appears that either the large chromosomes of an ancestral species have fragmented to produce the many small chromosomes seen in *M. reevesi*, or tandem fusion has occurred in an ancestral species to produce the large chromosomes present in *M. muntjac* (Gibson, 1984).

Still another type of variation in chromosome number occasionally in mammals, and more frequently in birds and reptiles is the presence of extra, often very small chromosomes, called B chromosomes or supernumerary chromosomes (Gibson, 1984). The Bs of some species are known to affect the frequency and distribution of chiasmata and consequently the phenotypic variation (Parker *et al.,* 1990). Their origin is unknown, but they may be remnants of chromosomal rearrangements (White, 1973). The presence of supernumerary chromosomes would change both chromosome number and the arm number, but they are generally counted separately.

If the position of the centromere is changed, as in a pericentric inversion (an inversion in which the centromere is included), the number of arms but not the chromosome number may be changed. An acrocentric chromosome may be converted to a metacentric chromosome, or the reverse may happen. Pericentric inversions have also been proposed to have occurred in speciation of bats (Baker and Bickham, 1980).

Some related species differ in arm number, but not in chromosome number because of the presence or absence of chromosome arms made of heterochromatin. The extra heterochromatic arms appear to be inactive genetically, and their origin is unknown. For instance, some populations of the burrowing rat-like animal, pocket gopher (*Thomomys bottae),* differ from other populations in having extra arms on some of their chromosomes (Patton and Sherwood, 1982). The populations are interfertile. When heterochromatin blocks are found interstitially, reproductive success may be affected. Although the polymorphism of heterochromatic regions have been observed as a normal variant in human, the high frequency of chromosomal variants in infertile men could support the option that a large heterochromatic block may destabilize the pairing of chromosomes and cause meiotic arrest, resulting in infertility (Lissitsina *et al.,* 2003).

Chromosomal evolution in the family Vespertilionidae is characterized by the conservation of entire chromosomal arms and reductions in diploid chromosome number via Robertsonian fusions. Less frequently, centric fissions, para and pericentric inversions and centromere shifts were found to have occurred (Volleth *et al.,* 2001). In several cases, a certain type of chromosomal change predominates in a karyotype.

2.7.2 Mechanisms of Change in Chromosome Morphology

Some of the morphological features of chromosomes are chromosome shape and length, centromeric position, telomeres, satellites, and nucleolar organizer regions. Cytogenetic analyses are almost always based on examination of chromosomes fixed during mitotic metaphase. During that phase of the cell cycle, DNA has been replicated and the chromatin is highly condensed. Metaphase chromosomes may differ from one another in size and shape, and the absolute length. However, the relative position of the centromere is constant, which means that the ratio of the lengths of the two arms is constant for each chromosome. One mechanism, which has been proposed as important in rapid speciation, is changes in chromosome structure (White, 1978).

Chromosomes may change in other ways, with no change in either the chromosome number or the arm number. A translocation occurs when a piece of one chromosome breaks off and attaches to another chromosome. Ordinary translocations between unlike chromosomes result in a change in shape of the chromosomes involved, but not in the number of chromosomes or chromosome arms.

A pericentric inversion (an inversion of a part of one arm) will change the order of genes on a chromosome without changing the size or shape of the chromosome. Differences between species due to a paracentric inversion appear to be uncommon in mammals, but such inversions have been proposed as a factor in speciation of bats (Baker and Bickham, 1980). Chromosome morphology can play an important role in the study of convergent, parallel and general evolution in bats when viewed in light of available data from all other evolutionary and taxonomic studies.

2.7.2.1 The Centromere

Each eukaryotic chromosome has a dense constricted area called a centromere (primary constriction) that serves as a point of attachment for spindle fibers during cell division. Generally, in mammals, centromeres contain repetitive DNA sequences, but these sequences vary substantially from one species to another. Four classes of chromosomes are generally recognized with respect to centromere position. Metacentric chromosomes have their centromeres at the middle. Submetacentric chromosomes have their centromeres slightly off center, such that one arm is shorter than the other. Acrocentric chromosomes have their centromeres close to an end, but not at the end whereas telocentric chromosomes have their centromeres located at the ends.

Based on the relative position of the centromere, the ratio of lengths of the two arms (r = 1/s where r =arm ratio, 1= long arm and s= short arm) allows classification of chromosomes into many basic morphologic types: median point (M), median region (m), submedian region (sm), subterminal region (st), terminal region (t) and terminal point (T) (Levan *et al.,* 1964). The corresponding arm ratios, respectively, are 1.0,1.0-1.7,1.7-3.0, 3.0-7.0, 7.0-∞, and ∞. The relative position of the centromere is constant, which means that arm ratio is constant for each chromosome. This ratio is an important parameter for chromosome identification.

2.7.2.2 Chromosome Length

Chromosomes differ greatly in size. Between organisms the size difference can be over 100 fold, while within a species some chromosomes are often ten times as large as others (Weaver and Hedrick, 1997). Chromosome size, including the total DNA content of the nucleus which is designated as absolute chromosome size, may vary as much as 20 – fold between genera of the same family having the same or similar basic chromosome numbers (Stebbins, 1971). Differences in absolute chromosome size between related species or genera probably reflect different amounts of gene duplication, either in tandem fashion or through polytene multiplication of chromonemata. Differences in relative chromosome size can be brought about by segmental interchange involving translocations of unequal size (Stebbins, 1971).

Deficiency or deletion makes chromosome shorter while duplication makes chromosome longer. Deletion is a case in which a chromosomal segment or gene is missing. This can be interstitial or terminal. Duplication is a situation in which a chromosomal segment or gene is represented more than once per haploid genome (Weaver and Hedrick, 1997). One can categorize duplications by the position and order of the duplicated region. Translocation is the movement of a chromosomal segment from one chromosome to another, nonhomologous chromosome. Obviously, translocations can change both the size of chromosomes and the position of the centromere.

The absolute length of any one chromosome varies depending on the stage of mitosis in which it was fixed. The efficiency of mitotic arresting agents varies, depending on the identity of spindle inhibitor, its concentration and duration of treatment, and even more on the cell type and its species of origin. The exposure time to spindle disrupting agent differs depending on the proliferative activity of the cells. Prolonged exposure to the drug or the use of high concentrations increases the proportion of chromosomes at late metaphase, resulting in shortening of the chromosomes. In addition, the concentration and type of salt used during hypotonic treatment are the main determinants of the average chromosome length in standard preparations (Ronne, 1989).

The type of fixative used is also another factor influencing chromosome length. After formaldehyde fixation at a relatively high pH, the chromosomes are thinner and longer (two to six times) when compared with chromosomes following methanol acetic acid fixation (Dietrich, 1986). Since the DNA content in mammals is relatively constant, chromosome size is inversely proportional to chromosome number (Fredga, 1977).

Garlova and Garlov (2000) tested a hypothesis on the selective neutrality of relative lengths of chromosomes. It is suggested that karyotype variation is limited by selection against chromosome rearrangements that produce very long or very short chromosomes. According to Garlova and Garlov (2000) chromosome rearrangements tend to randomize relative chromosome lengths in a karyotype, whereas natural selection acts to equalize them.

2.7.2.3 Secondary Constriction, Satellites and Telomere

Some chromosomes have secondary constrictions. The number of secondary constrictions in the chromosome complement agrees closely with the number of nucleoli. The secondary constrictions are nucleolar organizer regions (NORs). NORs were initially defined as sites around which nucleoli reorganize during telophase-interphase (Howell, 1982). During metaphase, these sites contain undercondensed DNA (Saitoh and Laemmli, 1994) identified as rDNA genes that are responsible for 80 % of total RNA synthesis within all the eukaryotic cells (Thiry and Goessens, 1996). Different organisms have different number of nucleolar organizer chromosomes, and the position of NORs is highly specific in a chromosome set. The part of the chromosome, distal to the secondary constriction, is sometimes very small and is known as a satellite.

The telomeres of chromosomes are composed of specific tandem repeats of the DNA sequence (TTAGGG)n that are conserved in all species of vertebrates (Meyne *et al.,* 1990). This repeated sequence has been detected not only in telomeres, but also in interstitial and centromeric chromosomal regions, in a variety of vertebrate species and in almost all of them, it is coincident with positive C-bands (Go *et al.,* 2000). Telomere sequences vary from species to species, but are generally GC-rich (Joeng *et al,*. 2004).

2.7.3. Chromosome Banding

Metaphase chromosomes can be classified via several methods, one of which, is by their banding patterns when stained prior to microscopic examination. Certain chemical treatments of chromosomes of amniotic vertebrates including mammalian chromosomes yield characteristic patterns of horizontal bands. The patterns obtained depend on the treatment used. A band is defined as that part of a chromosome which is clearly distinguishable from its adjacent segments by appearing darker or brighter with one or more banding techniques. The reproducible patterns of light and dark bands serve as signposts to identify different positions on chromosome. Bands do not represent single genes, but in fact the thinnest bands contain over a million base pairs and potentially hundreds of genes. With staining methods, it is possible to subdivide chromosomes into individual bands, each with a characteristic location, size and staining intensity (Fredga, 1977). Banding techniques reveal four main classes of bands (Verma and Babu, 1995): heterochromatic bands (C-banding), euchromatic bands (G-,Q-,and R-bandings), all of which are characterized by several molecular and functional features (Sumner,1994).

The banding techniques fall into two principal groups 1) those resulting in bands distributed along the length of the whole chromosome, such as G-,Q-and R-bands and 2) those that stain a restricted number of specific bands or structures. The latter include methods which reveal constitutive heterochromatin regions (C-banding), and nucleolar organizer regions, NORs (silver staining).

Applications of banding include chromosome identification and the determination of chromosome rearrangements in malformation syndromes and cancer, the chemistry of chromosome segments, chromosome changes during evolution, and, in conjunction with cell hybridization studies, chromosome mapping. Banding also permits the recognition of chromosome deletions, duplications and other types of structural rearrangements of chromosomes.

C-banding reveals constitutive heterochromatin. Constitutive heterochromatin exists in all mammals, replicates late during the S-phase, contains highly repeated DNA sequence, usually centromeric in location, highly polymorphic in size, and probably never transcribed (Hsu, 1975). C-banding is useful to show chromosomes with multiple centromeres and polymorphic regions that differ among individuals. The polymorphism is not only in size but also in the localization of the heterochromatin, and apparently has no phenotypic effect (Brown *et al.,* 1980).

The result of G-banding is alternating dark band (G-band or G-positive band) and light band (G-negative band). The dark regions tend to be late-replicating and AT rich whereas the light regions tend to be early-replicating and GC rich. The former corresponds to most Q-bands. Comparisons of G-banded chromosome patterns were first used to infer homologies of whole chromosomes or subregions between species and even across mammalian orders (Dutrillaux and Couturier, 1983). The band pattern is similar for homologous chromosomes but is different for nonhomologues.

Q- banding produces fluorescent bands (Q bands)which are brightest in AT-rich regions of the chromosomes (Hsu, 1979 in Hillis *et al* 1996), but also are influenced by variation in protein composition of the chromosomes (Benn and Perle, 1986 in Rooney and Czepulkowski, 1992). The pattern of bands is generally similar to that of G-banding. R-banding results in a banding pattern that is the reverse of G-banding or of Q-banding. Housekeeping genes are found in R-bands, which are GC-rich and replicate early in S-phase.

At metaphase, silver nitrate stains nucleolus organizer regions that have been active in the preceding interphase. NOR-banding is useful for identification and distribution of NORs in the chromosome complement and in clinical practice to study certain chromosome polymorphisms, such as double satellites.

2.7.4. Utilization of Karyotype Data in Bat Taxonomy

The development of various techniques in chromosome staining such as banding techniques allows the identification of homologs and homoeologs. Once homologs are identified, the chromosomes can be arranged as a karyotype by either cutting out photographic prints of chromosomes and pasting the homologs in pairs on white cardboard or using computer programmes developed for this purpose. A size-organized set of photographs of chromosomes is called a karyotype. Most animals have chromosome complements, karyotypes, which are characteristic for the species to which they belong. In some cases the same karyotype characterizes several genera or even families. For example, in North American *Myotis*, fifteen species had identical karyotypes of 2n=44, FN=50 (Baker, 1970 in Wimsatt, 1970). At the other extreme there seems to be considerable karyotypic variation in the genus *Pipistrellus*. The diploid number ranges from 26 to 44, with the FN ranging from 44 to 56 (Baker, 1970 in Wimsat, 1970). However, some members of the genus *Pipistrellus* have a karyotype very similar to that reported for most species of *Myotis*. Chromosome races seem to be less common in bats than in rodents. In no case more than two chromosomal races been reported for a species of bat (Baker, 1970 in Wimsatt, 1970).

In a comparison of four families of bats, involving 78 species (Baker and Bickham, 1980), Robertsonian fusions and pericentric inversions were dominant, with tandem fusions uncommon, and heterochromatin arms very rare. Intergeneric evolutionary relationships were studied in vespertilionid bats using G-and C-band data. Bickham (1979) suggested that karyotypic data of the family Vespertilionidae show 3 patterns of variability among genera. Some genera have retained the more primitive condition of high chromosome number, including *Myotis, Miniopterus, Eptesicus, Antrozous, and Nycticeius*. A second trend has been to become karyotypically more specialized by reducing the chromosome number through centric fusions, including *Plecotus, Idionycteris, Pipistrellus, Rhogeessa,* *and Lasiurus*. The third trend is seen only in *Lasionycteris* where the chromosome number has been greatly reduced through both centric fusions and translocations.

The 3 *Myotis* species in Taiwan have a distinctive external morphology and belong to different subgenera, i.e., *M. formosus watasei* belong to the subgenus chrysopteron, *M.latirostris* to selysius, *and M.taiwanensis* to leuconoe (Corbet and Hill, 1992). However, all have the standard of *Myotis* karyotype 2n=44 and FN=50(Baker and Patton,1967). No differences were found in chromosome morphology among the three species (Lin *et al.,* 2002). This fact suggests that the karyotype is an extremely stable feature in these bats, whereas the external characters are much more plastic (Lin *et al.,* 2002).

Comparing *Plecotus taivanus* with other Asian *Plecotus* species, the karyotype of this species is similar to those of *P.auritus sacrimontis* of Japan (2n = 32 and FN = 50, Ono and Obara, 1994) and *P.austriacus* of Europe (2n = 32 and FN=50; Fedyk and Fedyk, 1970). The karyotype of *Plecotus* has been regarded as having been derived from a *Myotis*-like karyotype by centric fusion (Harada, 1988). *Lasionycteris* (2n=20) has the most distinctive karyotype in the family Vespertilionidae. It possesses five pairs of large autosomes that have evolved through a series of centric and tandem fusions (Bickham, 1979).

Contrary to the above conserved karyotypes, the bats in the genus *Pipistrellus* are known to exhibit interspecific variations in karyotype (Ono and Obara, 1994). For instance, the karyotype of *P.abramus* from Japan was reported as 2n=26, FN=44 by Takayama (1959), and the G-and C-bands were reported by Obara *et al.* (1976) and Ando *et al.* (1980). *Pipistrellus abramus* in Japan has been considered to be synonymous with *P. javanicus* (Koopman, 1993). The latter species ranges from Afghanistan, Pakistan and India to Indonesia, the Philippines, Korea and Japan (Bates and Harrison, 1997). However, the diploid chromosome number of *P. javanicus* from India is 2n=36 (Dulic, 1981) and from Malaysia is 2n=34 (Volleth *et al.,* 2001) and differs considerably from that of *P.abramus.* Furthermore, features of the cranium and the teeth indicate that *P. abramus* is not synonymous with *P. javanicus* (Yoshiyuki, 1989).

Arielulus torquatus was first described as *Pipistrellus* species based on its external characters and number of premolars (Lin *et al.,* 1997), and later described as a new species from Taiwan by Csorba and Lee (1999). The chromosomal complement of this species is comprised of 24 pairs of acrocentric chromosomes varying in size from medium to small, a medium submetacentric X, and a small acrocentric Y chromosome. According to Lin *et al.* (2002) *A. torquatus* and *Eptesicus serotinus* share the same formula of 2n = 50 and FN = 48, whereas, a difference in size of the Y chromosome was found. These authors support the views of Csorba and Lee (1999) and Volleth *et al.* (2001), who consider *Arielulus* to be closely related to *Eptesicus*.

3. OBJECTIVES OF THE STUDY

3.1. General objective

The general objective of this study is to investigate the chromosomes of some bat species occurring in Ethiopia.

3.2. Specific objectives

The specific objectives of this study are to:

- determine chromosome number of the studied bat species.
- describe chromosome morphology and characterize karyotype of the studied bat species.

4. MATERIALS AND METHODS

4.1. Collection of specimens

4.1.1 Study localities

Specimens were collected from four localities in Ethiopia (Fig.1). The geographic location, altitude of each locality and the number of specimens collected are presented in Table 3. The localities were selected with reference to their bat diversity and availability of suitable roosts (caves, trees and roof of houses).

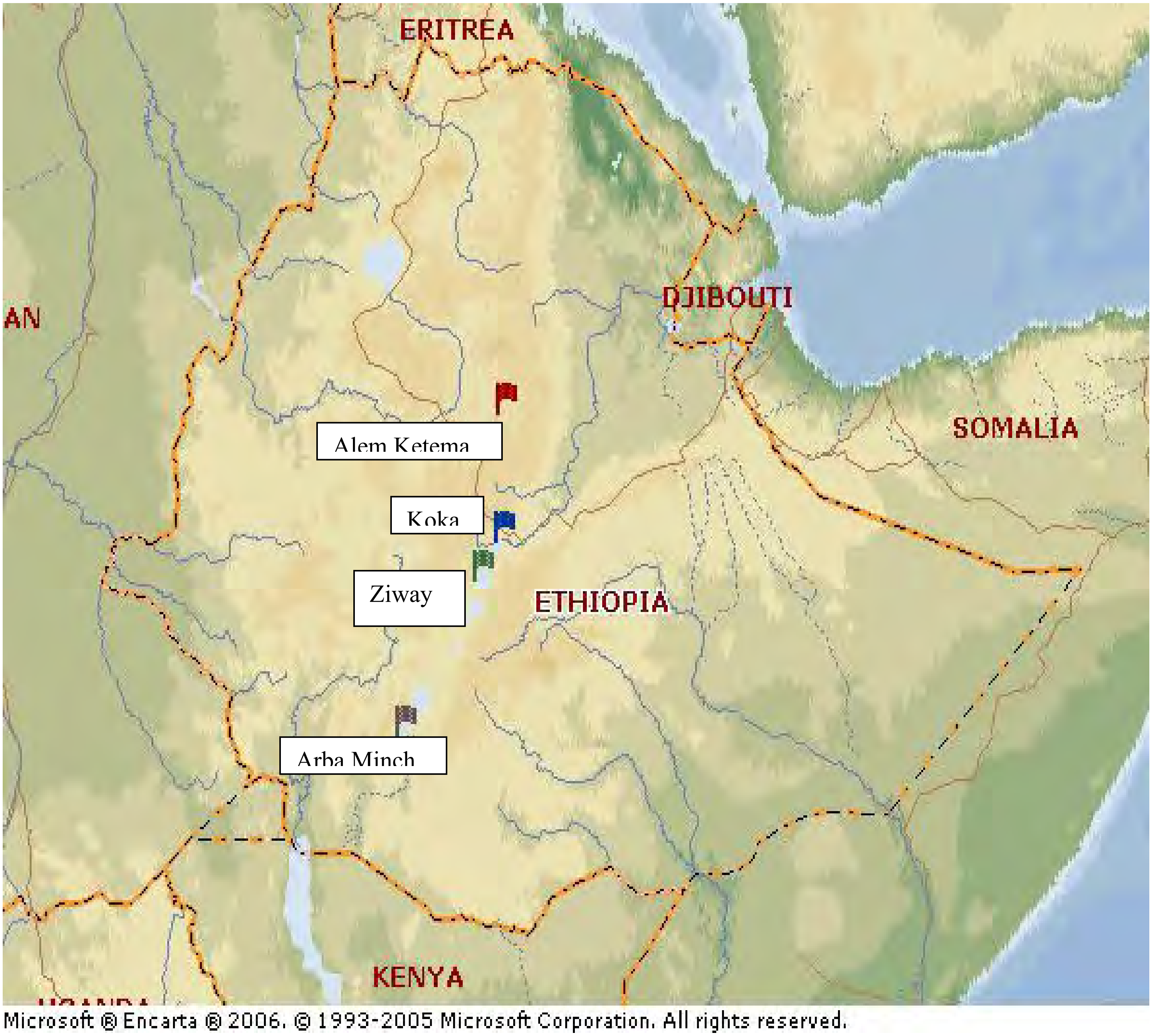


Fig .1. Map of Ethiopia showing sites of bat specimen collection sites for the present study.

Table 3. The species of bats, geographic location and the number of bats captured.

Species	Locality	Geographic location	Male	Female	Total
Chaerephon pumila	Ziway	$7^0$5'N, 38^0 42' E	1	1	2
	Arba Minch	$5^0$57' N 37^0 32' E	5	5	10
Micropteropus pusillus	Ziway	$7^0$5'N, 38^0 42' E	5	4	9
	Koka	8^0 26' N 39^0 01'E	2	1	3
	Arba Minch	$5^0$57' N 37^0 32' E	2	3	5
Pipistrellus pipistrellus	Ziway	$7^0$5'N, 38^0 42' E	2	2	4
Scotophilus dinganii/viridis	Ziway	$7^0$5'N, 38^0 42' E	1		1
DD5	Alem Ketema (Merehabete)	10^0 04' N 39^0 01'E	2	2	4
		Total	20	18	38

4.1.2. Method of Capturing

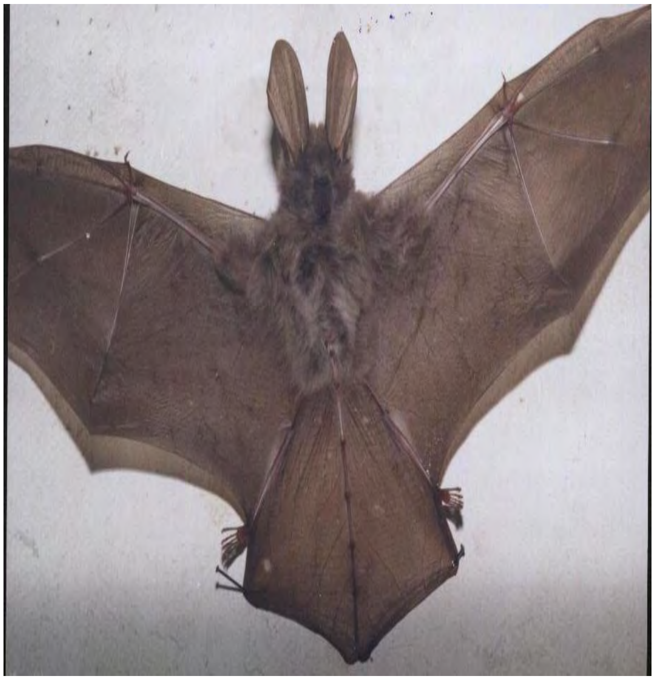
A large butterfly net was used at the exit of caves and roof of buildings in addition to large fishnets. Trapping with nets, spread out on frames or draped over escape holes was another method used to capture bats. Hand collecting method was also used in a banana plantation where the height of the banana plants was low enough to be reached. The photographs of some captured representative bats are shown in Fig. 2 (A-E).

A

B

C

D



E

Fig . 2. Specimens captured from different localities of Ethiopia. A, *Micropteropus pusillus.* B, *Pipistrellus pipistrellus.* C, *Scotophilus virids (S .dinganii).* D, *Chaerephon pumila* and E, Specimen from Merehabete region

4.1.3. Preservation of specimens

Each bat specimen was placed in a container with 70% ethanol. The container is tightly sealed to avoid weakening of the spirit due to evaporation. The preservation was made after the removal of the humerus for chromosome preparation from the bone marrow. The karyotyped specimens were properly labeled, preserved and kept in the Genetics Laboratory, at Science Faculty of Addis Ababa University for later deposition in the Natural History Museum, Department of Biology, Science Faculty of Addis Ababa University.

Alcohol of such strength is neither too weak to prevent decay nor too strong as to rob the specimen of all suppleness. Spirit material has certain advantages over stuffed skins to work upon (Rosevear, 1965), such as the retention of plasticity and the avoidance of excessive shrinkage and loss of shape. The drawbacks to spirit are that the specimens are more messy to handle, and that colours fade in it to an unpredictable degree (Rosevear, 1965).

4.2. Somatic chromosome study

4.2.1. Metaphase chromosome slide preparation

Chromosome preparations were made from bone marrow cells according to Lee and Elder's (1980) method cited in Hillis *et al.* (1996) with omission of injection of yeast suspension. Individual bats were weighed using a hand balance and injected peritonially with 0.05% colchicines at a rate of 0.1m1/10g body weight and left in a cage. About one and half hours later, the animal was sacrifised (anesthetized with diethyl ether followed by killing by breaking at the junction of the skull and vertebrae). The upper arm bones (humerus) were dissected and crushed, to release the bone marrow cells, in a Petridish containing about 5 ml of hypotonic solution of 0.075M KCl. Using Pasteur pipette, the cell suspension was transferred from the Petridish to a centrifuge tube, and incubated at room temperature for 20-30 minutes, centrifuged, and the supernatant was discarded. Then, freshly prepared 3:1 (methanol: acetic acid v/v) was added to the pellet, the tube flicked constantly and after 10 minutes of fixation it was centrifuged at 1000 rpm for 10 minutes. The supernatant was discarded and the tube was flicked vigorously to loosen the pellet. After a total of 3 rounds of suspension in fixative followed by centrifugation, the pellet was re-suspended in a small volume (<0.5ml) of fixative.

Test slides were prepared to check for cell density and presence of metaphase spread of chromosomes. When found necessary the cell density was adjusted by diluting or spinning down and resuspending the cells in a smaller volume of fixative. When metaphase chromosome spreads were observed, 8-10 slides were prepared, by splashing a few drops of the cell suspension on a glass slide from a height of about 0.5m or more. The slides were then air-dried and stored. Sometimes slides were prepared in the field using locally available facilities such as schools and agricultural bureau found near the site of collection. In some cases, live bats were brought to the Department of Biology and chromosome preparation was done in the laboratory at the Department.

4.2.2. Giemsa staining

Air dried slides were stained with Giemsa in phosphate buffer (pH 6.8) for 15 minutes or more as necessary. The slides were rinsed in two changes of distilled water, air dried at room temperature and mounted under a 22x50mm cover slip with Depex.

4.2.3. Karyotype Analysis

Photomicrographs of metaphase plates with good chromosome spreads were taken using a camera-fitted microscope with a magnification of x100 objective. Chromosomes were described and characterized using photomicrographs and direct observation under the microscope. The total length, the arm lengths and the arm ratios of the chromosomes were computed using a micromeasure computer program (Appendix 1-9 and Table 4). Chromosome size and centromere position were used to arrange putative homologous chromosomes into pairs to construct the karyotypes. The number of bats studied from each locality and the number of metaphase cells analyzed from each locality are shown in appendix 1.

5. RESULT

The total number of bats studied is 38 (Table 3).

5.1. Karyotype Description of *Micropteropus pusillus*

Somatic metaphase chromosome analysis has been made from 11 specimens of this species collected from Koka (KT_1-KT_3), Ziway(ZA_1-ZA_3,ZF_1-ZF_3 and ZKM) and Arba Minch(AT_3).Representative metaphase chromosome spreads (Figs.3) and karyotpes (Figs.4) are presented. Two chromosome numbers and two fundamental numbers have been observed. All the specimens have 2n=35 and FN=70. Occasionally however, cells with 2n=36 and FN=72 have been observed in the same specimens having 2n=35.

In both cases, all the chromosomes are biarmed which can be grouped into about 23 metacentrics and 12 submetacentrics. The metacentrics show continuous gradation in size from large to very small chromosomes. When arranged into pairs, these chromosomes form 11 pairs and the smallest chromosome is left with out a pairing partner. The 12 submetacetrics are consisting of nine medium sized and three small chromosomes. Arranging of the chromosomes into pairs reveals that one medium sized and one small chromosome will be left without pairing partners.

Chromosome complements with 2n=36 are presented in fig.3 and 4. The karyotype shows that the extra chromosome is the smallest chromosome,i.e.,the 36th chromosome.

Chromosomes from Arba Minch (AT3) and Ziway (ZKM) specimens are not presented due to lack of good cells with good chromosome spreads. However, visual analysis under the microscope has shown that they have similar karyotype as that of the rest of the specimens. The Karyotypic formula is 23m+12sm (Table 4).

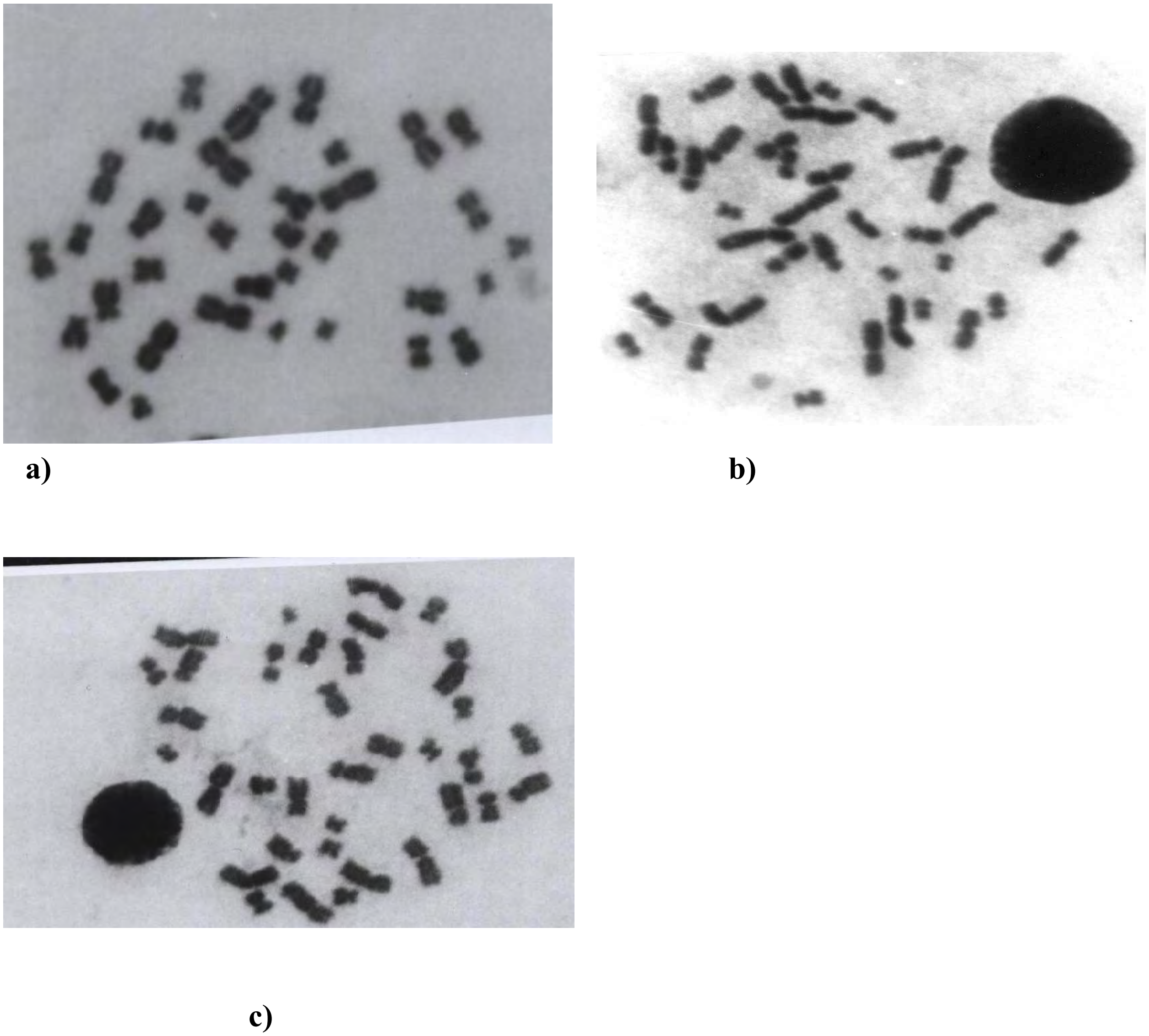
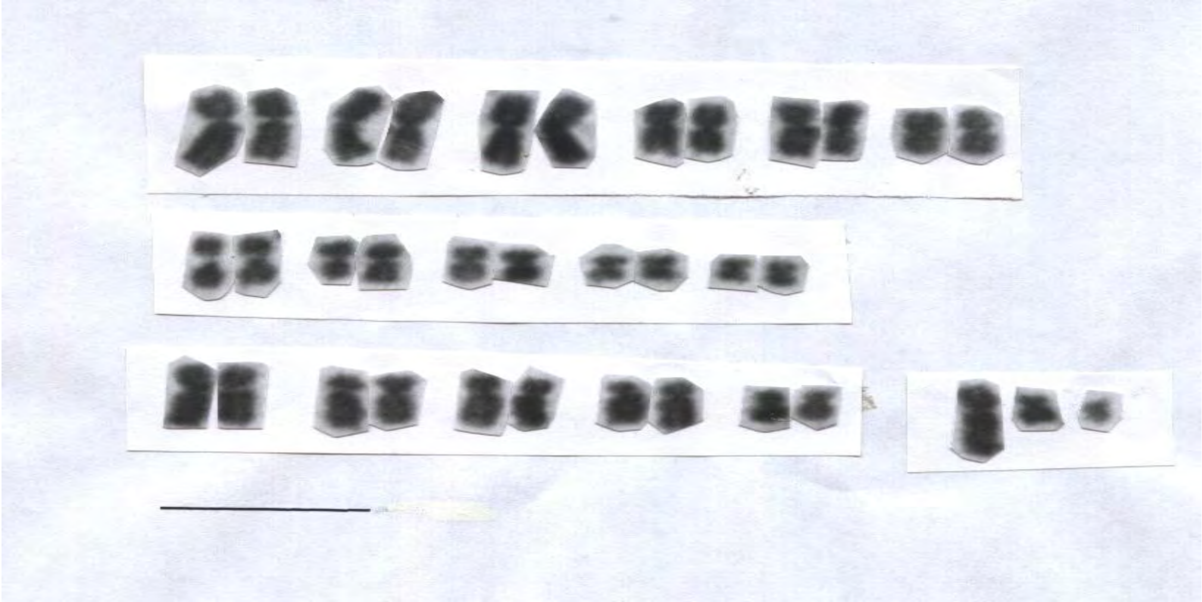
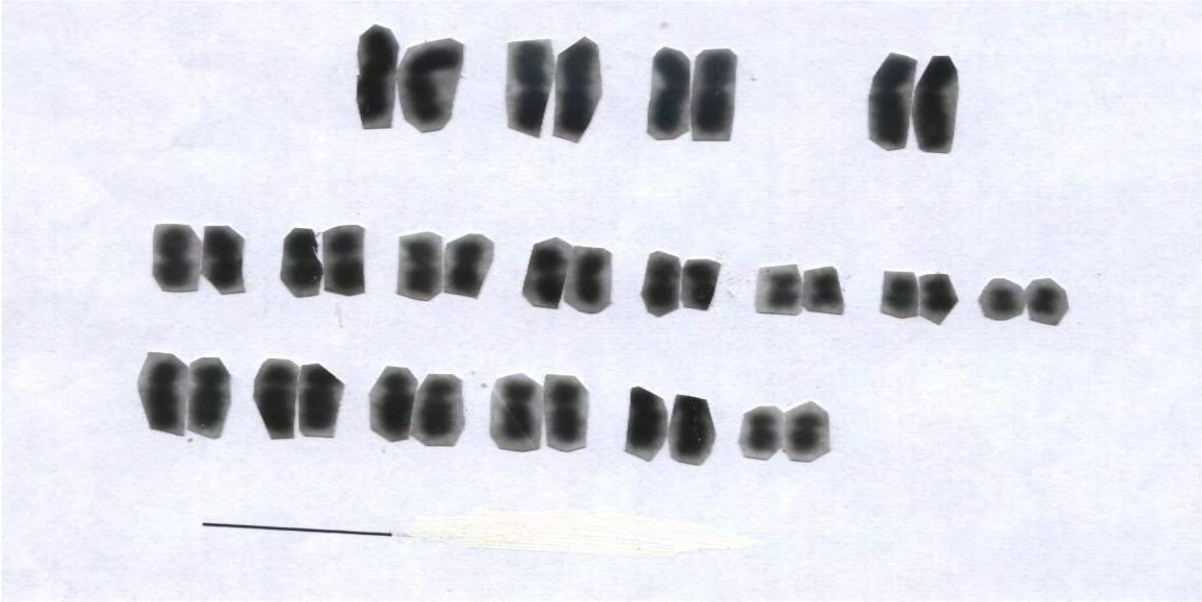


Fig.3. Mitotic metaphase chromosome spreads of *Micropteropus pusillus* specimens; a) male from Ziway (2n=35); b) male from Ziway(2n=36); c) male from Koka (2n=36).



a)



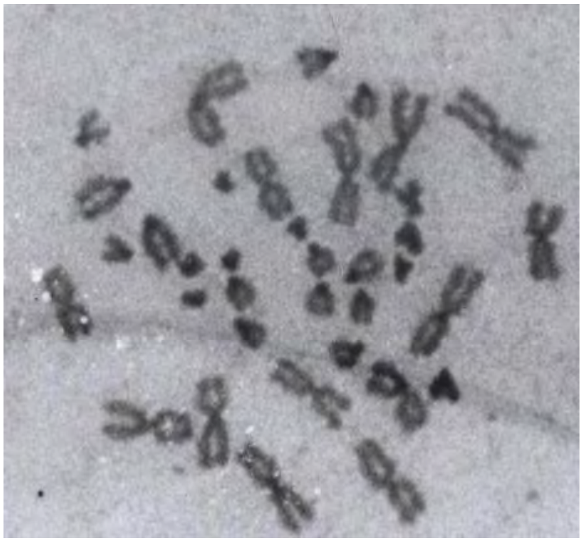
b)

Fig. 4 Karyotypes of *Micropteropus pusillus* specimens; a) from Koka with 2n=35 b) from Ziway with 2n=36. Bar = 2.4 µm

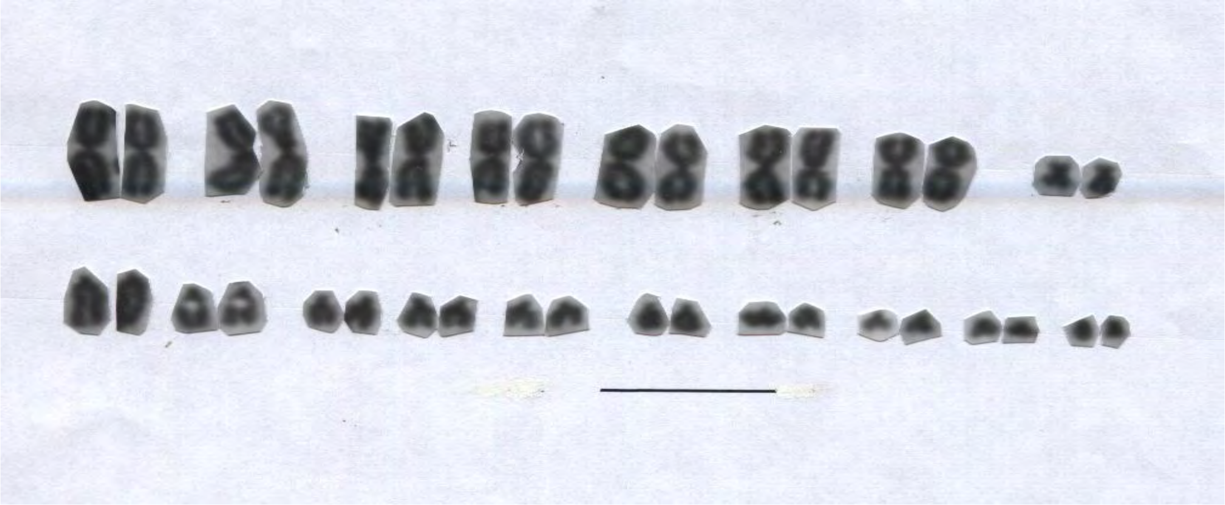
5.2. Karyotype Description of *Pipistrellus pipistrellus*

Three specimens Ziway, coded as ZC_2, ZC_3 and ZC_4 were studied. The mitotic metaphase chromosome spreads and karyotype are shown in Fig.5. They all had 2n = 36 and FN = 52. The chromosome complements consist of seven pairs of large metacentrics, one small pair of submetacentric and ten pairs of acrocentrics. The latter range in size from medium to a very small chromosome. The metacentric chromosomes are more or less of similar size with only slight gradation. Among the acrocentrics, the largest pair has the size about half that of the largest metacentiric. Although the acrocentric chromosomes show continuous variation in size, the first two pairs can easily be distinguished from all the rest (Fig. 5). The karyotypic formula is 14m+2sm+20t (Table 4).

One notable feature of the acrocentrics is that their centromeric regions stain darkly and several of the small acrocentics are often observed staining wholly stain dark, indicating their constitutive heterochromatin constituent (Fig. 5a). This differential staining has been produced by normal Giemsa staining without any pretreatment for C-banding. Similar staining has been observed in the centromeric regions of some of the metacentrics as well as the small submetacentric pair. This indicates that even the small arms of the latter are not heterochromatin.



a)



b)

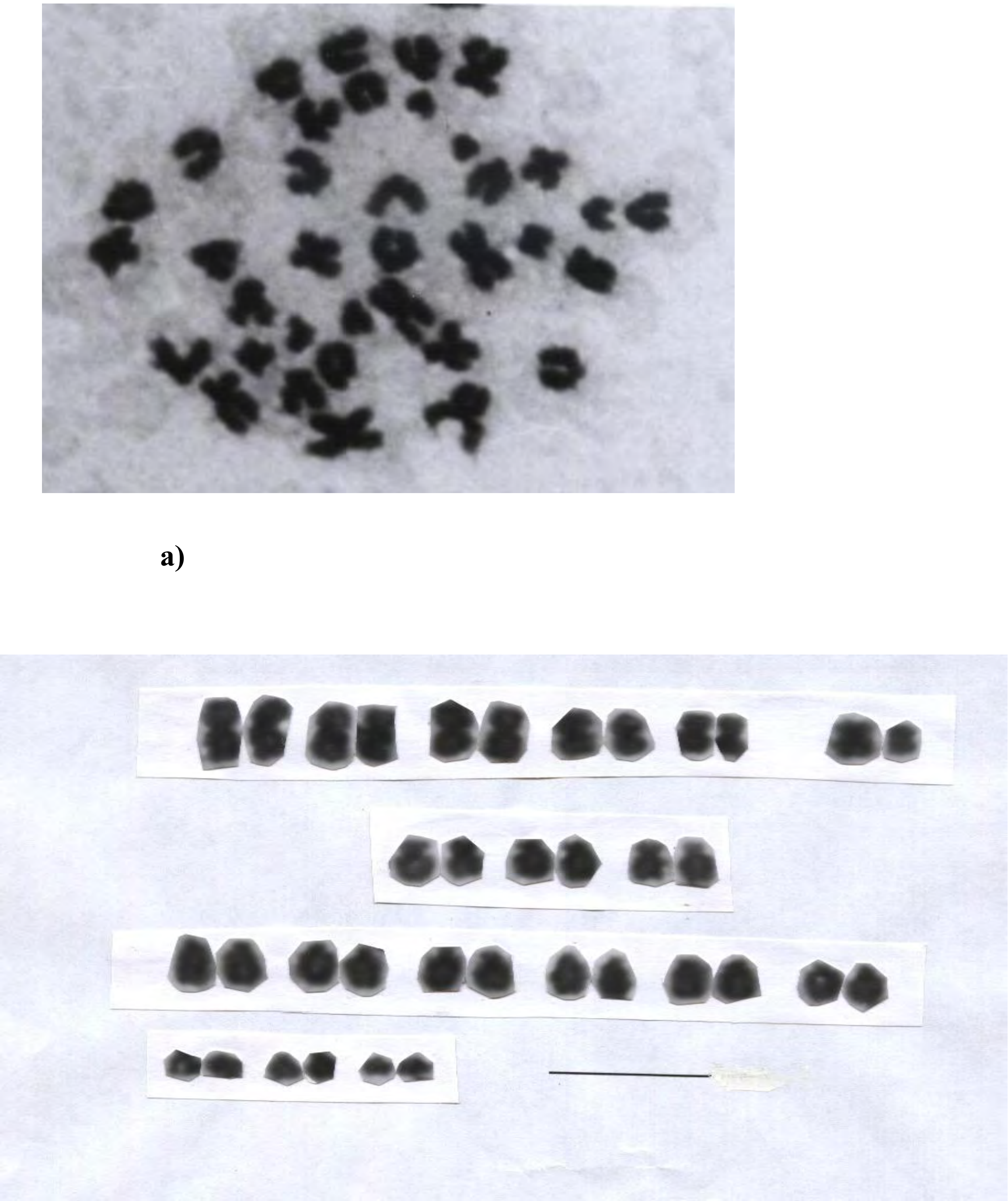
Fig. 5 a) Mitotic metaphase chromosome spread and b) karyotype of *P. pipistrellus* (female). Bar =2.4 µm

5.3. Karyotype Description of *Scotophilus* species

Only one specimen belonging to the genus *Scotophilus* was captured from Ziway. The specimen has been identified to be either *Scotophilus viridis* or *S.*

dinganii. The specific distinction needs comparison of cranial features, which has not been possible to do in the present study.

The specimen has 2n=36 and FN = 54 (Figs. 6). Six pairs of the chromosomes are metacentrics, three pairs are acrocentrics and nine pairs are telocentrics. One pair of the metacentirics shows size polymorphism and they probably constitute a pair of sex chromosomes. The acrocentrics fall into two size groups of six pairs of relatively larger and more or less of similar size and three pairs of small chromosomes which are also of similar size. The karyotypic formula is 12m+6a+18t (Table 4).



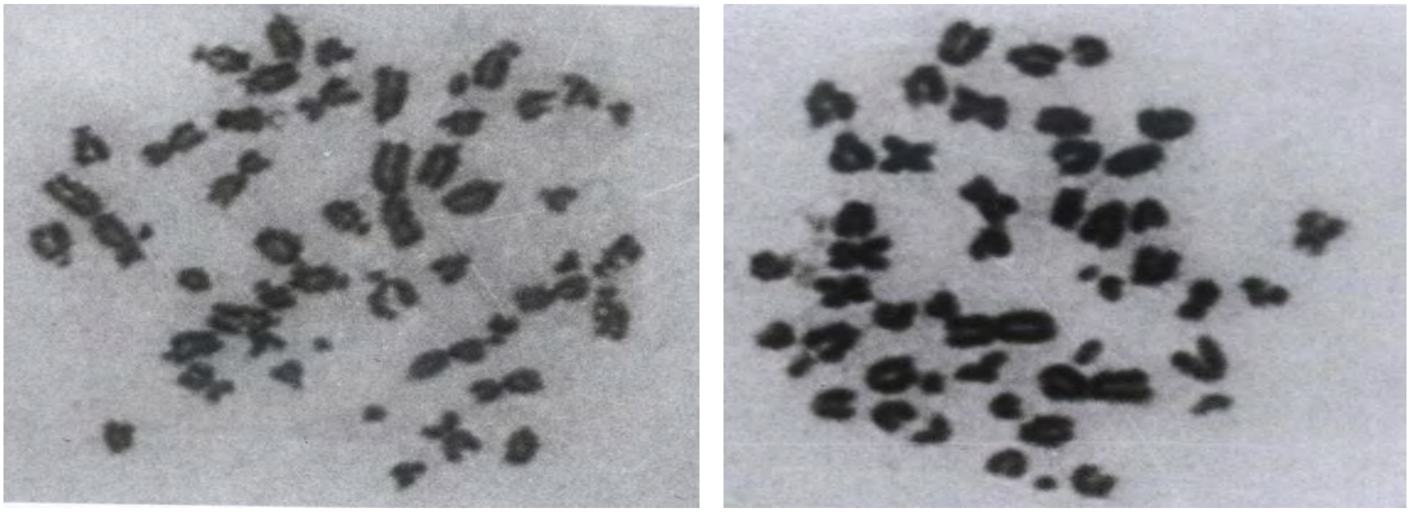
a)

b)

Fig. 6 (a) Mitotic metaphase chromosome spread and (b) karyotype of male *Scotophilus viridis* or *S. dinganii.* B=2.4µm

5.4. Karyotype Description of *Chaerephon pumila*

Specimens of this species were captured from Ziway and Arba Minch. Karyotype analysis was made from two specimens of Ziway (ZK_1 and ZK_2). The mitotic metaphase chromosome spread and karyotype are presented in Fig.7. They consist of 2n = 48 and FN=60. There are one large and four medium sized pairs of metacentrics. Eighteen pairs of telocentrics ranging from medium to small vary in a continuous manner. One additional medium sized metacentric and one small sized acrocentric have no pairing partner which could probably be a sex chromosome pair. Due to lack of good chromosome spread, karyotyping of chromosomes from Arba Minch specimens was not possible. However, visual analysis under the microscope shows that this specimen is chromosomally similar to the Ziway specimens. The karyotypic formula is 11m+1a+36t.



a) **b)**

Fig. 7 Mitotic metaphase chromosome spreads of *Chaerephon pumila* specimens from Ziway (2n=48) a) ZK_1 (male) and b) ZK_2 (Female)

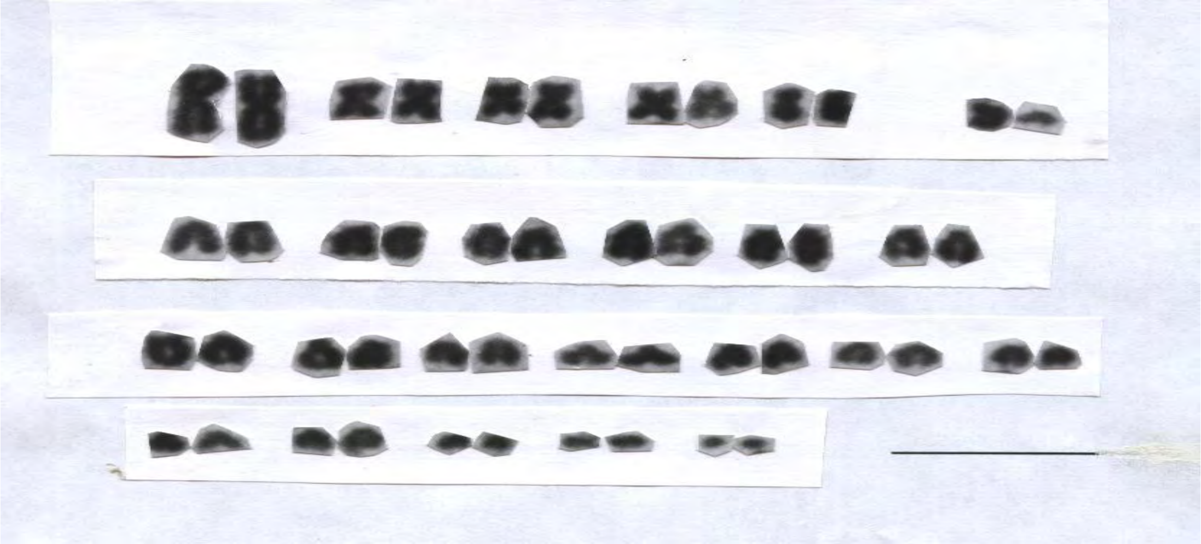


Fig. 8 Karyotype of male *Chaerephon pumila*. Bar = 2.4µm

5.5. Karyotype Description of Specimen from Merehabete region

A specimen given a code DD_5 was captured from a cave known as “Dim-Dim” near a village called Kum-Amba in the vicinity of Alem Ketema town in the Merehabete region. Taxonomic identification of this specimen has not yet been done due to lack of a bat taxonomist. Mitotic metaphase chromosome spread and karyotype of this specimen are presented in Fig.9, and consist of 2n=42 and FN =82. The karyotype is comprised of 20 pairs of medium sized meta and submetacentrics and the smallest pair of telocentrics. The karyotypic formula is 36m+4sm+2t (Table 4).

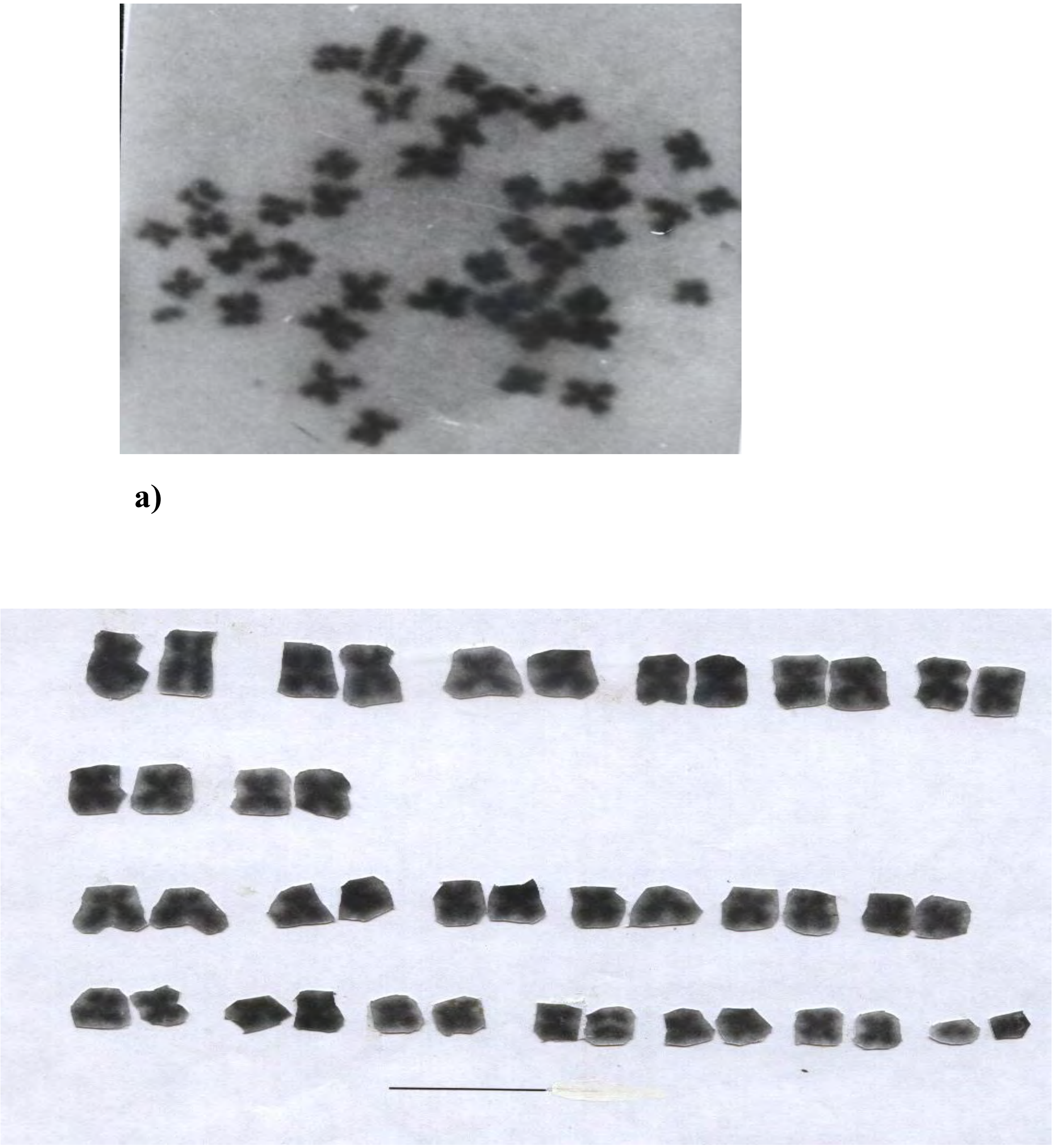


Fig. 9 a) Mitotic metaphase chromosome spread and b) karyotype of specimen from Merehabete. Bar = 2.4μm

Table 4. Karyological data of the studied bats from different localities in Ethiopia showing the different centromeric positions (M, m, sm, st, t and T), and the diploid and fundamental numbers.

Specimen code	M	m	sm	st	t	T	2n	FN
ZA_1		23	12				35	70
ZA_2		24	12				36	72
ZF_1		23	12				35	70
KT_1		23	12				35	70
KT_2		23	12				35	70
KT_2		24	12				36	72
ZC_3		14	2			20	36	52
ZB_2		12		3	3	18	36	56
DD_5		36	4			2	42	82

N.B. M and m = metacentric; sm = submetacentric; st and t = acrocentric; T=telocentric.

6. DISCUSSION

The chromosomes of bats from four different localities in Ethiopia were studied and five different karyotypic forms were found. The bats belong to 3 families and 4 genra plus one specimen not yet taxonomically identified. *Pipistrellus pipistrells* Schreber and *Scotophilus dinganii* or *S. viridis* belong to family Vespertilionidae; *Micropteropus pusillus* Peters to Pteropidae; C*haerephon pumila* Cretzchmar to Molossidae.Taxonomic identification of the specimen from Merehabete region has not yet been done.

The karyotype of *M. pusillus* shows two forms. The specimens from both Koka and Ziway (KT_1, KT_2, ZF_1 and ZA_2) are characterized by having 2n=35 and FN=70. The other specimens from the same localities (KT_2, ZA_2 and ZKM) and from Arba Minch (AT_3) possess additional karyotype of 2n=36 and FN=72. The former karyotype is the most frequent with rare observation of the 36th chromosome. In 2n = 35, the odd number may be explained by the presence of XY_1Y_2 sex chromosome constitution. The diploid number of the first category, i.e., 2n=35, is in agreement with results of Haiduk *et al.* (1980 and 1981) whereas the diploid number of the second category is different from that reported by these authors.

Baker (1970) attributes numerical variation of chromosomes between plates within an animal to technical errors and assumes that there is no mosaism of chromosome numbers within individuals in bats. However, our observation is at variance with this assumption. We have observed metaphase plates with 2n = 36, though at low frequency, along side with the majority of plates of 2n = 35 chromosomes (Fig.3). As this mosaism has been observed only is *M. pusillus*, not in other species whose chromosomes, were studied by the same technique, the observed mosaism could not be attributable to technical error. There should be some other factor(s) responsible, which needs future investigation. One possible tentative explanation could be anaphase non-disjunction of sister chromatids whereby a cell can receive an extra copy of the chromosome involved in non-disjunction.

The two karyotypic forms of *M. pusillus* also differ in fundamental number which is a consequence of the difference in diploid number. The fundamental number of the first category is in agreement with that reported by Haiduk *et al.* (1980 and 1981) while the fundamental number of the second group is different from that reported by these authors. In both forms, all the chromosomes are biarmed, hence, the karyotype is largely symmetrical.

The karyotype of *P. pipistrellus,* comprising of 8 pairs of biarmed and 10 pairs of medium to small-sized telocentric chromosomes form a bimodal karyotype. The diploid and the fundamental numbers were 36 and 52, respectively, described in this study are different from 2n=42 and FN=50 reported for the species (Bovey, 1949 in Baker, 1970; Fedyk and Ruprecht, 1976; Volleth, 1987). However, the 2n value observed in this study is within the range of 2n values 26-44 reported for the genus (Bickham, 1979) while the FN value is above the range of FN values 44-50 reported for the genus by the latter author.

The telocentric chromosomes of *P. pipistrellus* are heterochromatic entirely or at least at the centromeric region. There are similar reports of heterochromatin localization in centromeric region of bat chromosomes. For instance, all autosomes of *Pipistrellus abramus* from Taiwan contain certromeric constitutive heterochromatin with especially large heterochromatin blocks in the pericentromeric regions of chromosomes 1 through 4, 10 and 11 (Lin *et al.,* 2002). The X chromosome also had a block of heterochromatin in the pericentromeric region (Lin *et al.,* 2002). In chromosomal studies among nine species representing six genera of the family Emballonuridae, heterochromatin was restricted to centromeric regions in most taxa (Hood and Baker, 1986). However, in this family *Rhynchonycteris naso* has an interstitial heterochromatin on the largest acrocentric autosome, and *Balantiopteryx plicata* and *Cormura brevirostris* have incorporated large amounts of heterochromatin in their autosomal complements (Hood and Baker, 1986).

The chromosome complement of *P. pipistrellus* is asymmetrical and consists of two sharply distinct classes of chromosomes, large metacentrics and small telocentrics. This has been termed as bimodal karyotype. According to Levitzky's principle of increasing karyotype asymmetry, bimodal karyotypes could result from unequal translocation, by means of which certain chromosomes would periodically contribute segments to others of the same complement (Stebbins, 1971). The size of donor chromosomes would thus become reduced, and that of recipients correspondingly increased. Although centric fissions are less frequent in chromosomal evolution in the family Vespertilionidae (Volleth et al., 2001), some bimodal karyotypes may result from metacentric complements if some of the chromosomes undergo Robertsonian fission to form telocentric chromosomes. Conversely, if the ancestral karyotype is consisting of telocentrics, bimodal karyotypes of this sort can arise in the desendants through Robertsonian fusion of some of the telocentrics into metacentric. Robertsonian fusion and fission are of frequent occurrence in the karyotype evolution of mammals with telo or acrocentric chromosomes.

The chromosome complement of *S. dinganii* (*S. viridis*) is characterized by 2n=36 and FN=54. The diploid number is in agreement with that reported for the species by Schlitter *et al.* (1980), whereas the fundamental number is different from 56 for *S. dinganii* and FN=58 for *S.viridis* reported by these authors. The description of the pair of metacentrics that are polymorphic in size as sex chromosomes is different from that reported by Schlitter *et al.* (1980). According to these authors X is acorocentric while Y is metacentric. Although the karyotype is composed of 8 pairs of metacentric and 10 pairs of acrocentric or telocentric chromosomes, with the exception of chromosomes 35 and 36, all the chromosomes are medium-sized (Appendix 8). Therefore, the chromosomes are comparable to each other in size. This is termed as homogeneous karyotype (Stebbins, 1971).

It has not been possible to determine whether the specimen of genus *Scotophilus* used in the present study is *S. dinganii* or *S. viridis* from external morphology since the distinguishing features lie within cranial skeletal features. Our chromosome data could help not in this regard either. The 2n = 36 has been reported for both species (Schlitter, *et al.*, 1980) which is in agreement with our finding for the present specimen. On the other hand, all the three differ in their fundamental numbers, FN = 54, FN = 56 and FN = 58, for the present specimen, *S. dinganii* and *S. viridis*, respectively. Whether the present specimen belong to one of the two species or a different species need, further detailed studies involving chromosomal and morphological characteristics.

The karyotype of *Chaerephon pumila* is composed of 11 metacentrics and 37 acrocentric or telocentric chromosomes. The description of the medium-sized metacentric and the small-sized acrocentric as sex chromosomes is in agreement with metacentric X and acrocentic Y (Dulic and Mutere, 1973 cited in Baker and

Bickham, 1980). The chromosome set is considered as asymmetrical karyotype. It is known that telocentrics can be derived by mis-division of meta or acrocentrics but there is no need to assume that all are necessarily derivative (Jones, 1970). Increasing asymmetry results from pericentric inversions and unequal translocations of portions of chromosome arms. It may, therefore, take place without changing the number of centromeres or of independent chromosomes (Stebbins, 1971).

The diploid and fundamental numbers of *C. pumila* are 48 and 60, respectively. The 2n number is in agreement with that reported by Dulic and Mutere, 1973 cited in Baker and Bickham, 1980 while the FN is different from FN=62 reported by these authors. The proportion of acrocentrics or telocentrics is 70.7% of the chromosome set. Compared with the proportion of the chromosome number, the fundamental number is low. This might be expressed in the following manner. By converting metacentric to acrocentric chromosomes, pericentric inversions can reduce the fundamental number of well developed chromosome arms (Stebbins, 1971).

The chromosome set of the specimen from Merehabete (DD5) possesses 20 pairs of biarmed and a pair of telocentric chromosomes. Being composed of mainly biarmed chromosomes, the karyotype is symmetrical and in terms of chromosome size it is homogeneous. There are two possible explanations for this. In the first case, this may not be derivative, thus it might stem from some symmetrical karyotype of a remote ancestor. Another possibility is that metacentric chromosomes can arise by the fusion of two telocentrics, without any material alteration of either the chromosomal contents or the arrangement of genes (White, 1973). These changes are apparently common in the evolution of mammalian species (Fredga, 1977).

The diploid and fundamental numbers of the specimen from Merehabete region (DD_5) are 42 and 82, respectively. Compared with the mean FN, i.e, 51.6 reported for bats (Baker, 1970 in Wimsatt, 1970). The fundamental number is high. The possible explanation for this may be as follows. Centric fusions between acro or telocentric chromosomes to give metacentric chromosomes always consist of the transfer of whole arms. Consequently, they inevitably produce a reduction in the number of centromeres and chromosomes, while leaving the fundamental number of arms unchanged (Weaver and Hedrick, 1997). The role of Robertsonian rearrangement in the evolution of bat karyotypes has been discussed by several authors, most of whom agree that centric fusion is the most frequently occurring (Baker, 1970).

The chromosome complements described in this study can be classified into two categories: symmetrical and asymmetrical karyotypes. It is evident that numerous chromosomal changes have occurred since the karyotype of the common ancestor of bats (Baker, 1970). There are two trends in karyotype evolution; symmetrical karyotype preceeds asymmetrical karyotype (Jones, 1970) or asymmetry gives way to symmetry (Vaarama, 1954). In spite of the occurrence of the two karyotypic forms, the direction of the change cannot be determined on the basis of these data.

According to Baker (1970, in Wimsatt, 1970) centric fusion is the most frequently occurring chromosomal rearrangement in the evolution of bat karyotypes, therefore, the symmetrical karyotypes observed in this study may be derived from asymmetrical karyotypes. However, there is a need to consider that these chromosome sets may not be derivative and thus they might stem from some symmetrical karyotype of a remote ancestor. On the other hand, the asymmetrical karyotypes may be evolved from symmetrical ones since numerous acrocentrics may be formed from biarmed chromosomes by centric fission but there is no need to assume that all are necessarily derivative.

Another considerable discrepancy is that the diploid and fundamental numbers of *P. pipistrellus* are different from that reported by several authors (Bovey, 1949 in Baker, 1970; Fedyk and Ruprecht, 1976, Volleth, 1987). Chromosome feature is certainly an independent character from cranial features and gross anatomy (Baker, 1970). The latter two characters are strongly affected by adaptation to different feeding niches. For this reason and because bat chromosomes seem to evolve at a slow rate when compared to the rate of change in gross anatomical features on which present phylogeny is constructed, similarities or divergence in bat karyotypes should receive serious consideration as indicators of degrees of relationship between forms under consideration (Baker, 1970).

In Ethiopia, bats are critically threatened by encroaching agriculture and collection of firewood resulting in loss of roosts; and insecticides and pollution which diminish the bat's food supply. In two of the localities of this study, Arba Minch and Ziway, bats roosting in ceilings of schools and stores, are exterminated indiscriminately for bad odour in buildings where they roost (personal observation). In Ziway, it is also known that bats have been hunted for preparing pseudomedicine (personal observation). Another factor, which contribute to the declines in number of bats, is a severe lack of detailed knowledge about the ecological requirements of most bat species.

7. CONCLUSION

Chromosomal differences reflect differences in the source of genetic variation, while morphological, physiological, and biochemical differences reflect differences in the products of gene action, modified by environmental influences.

The diploid numbers of *M. pusillus* of the present study are 35 and 36, the former being more frequent than the latter. The reason for this is unknown. Since chromosome numbers are important in karyosystematics, mechanisms that lead to stepwise numerical changes deserve particular consideration.

Many cryptic or sibling species are distinguishable on the basis of their karyotypes, they may be morphologically indistinguishable but biologically distinct.

The bimodal karyotype observed in *P. pipistrellus* may be derived from symmetrical karyotype by Robertsonian fissions or unequal translocation. On the other hand, it seems to be that in Vespertilionidae the acrocentric condition is primitive due to the remarkably high frequency of situations in which centric fusions are indicated (Bickham, 1979). Different organisms may evolve their chromosome complements in different ways and one may be quite unjustified in extrapolation from one group to another in the present study since the bats belong to 3 or 4 families. Although applying the concept of ancestral (primitive) and derived (advanced) relationship of the karyotypes is not possible, the data are valuable for future studies on karyostematics of these bats.

The variations observed in diploid and fundamental numbers signifies that these bats are diverse in their karyotypes. Since chromosomal rearrangements may alter the number of chromosomes, the number of chromosome arms, or both, chromosome repattering and dysploid change might have occurred in the evolution of the chromosomes of the studied bats if an ancestral karyotype is assumed for chiropterans.

The acrocentric or telocentric chromosomes of *P. pipistrellus* are heterochromatic either entirely or at least at the region of their centromeres. The difference in degree and distribution of heterochromatic regions is useful in identifying particular chromosomes. This landmark is, therefore, essential as a principal morphological characteristic of karyotypes. Hence, it provides vital data for karyosystematics of bats. However, investigating detailed homology relationship among the heterochromatic regions is highly unlikely in this study and calls for high resolution banding and other molecular techniques.

Gross anatomical features have been used to design the systematics and taxonomy and phylogeny of bats. There is not yet enough known about the ecology, physiology and behavior of these organisms. Moreover, little is dealt about their karyotype. Differences in chromosome structure are often correlated with taxonomic differentiation (Sessions, 1996 in Hillis *et al.,* 1996) This study shows the need to take the chromosome characteristic into consideration since it can provide valuable data for taxonomic and evolutionary studies on bats.

8. RECOMMENDATIONS

Based upon the study on bat chromosomes carried out at four localities of Ethiopia, the following recommendation can be made:

- Further studies using C-, G- and high resolution banding techniques may be useful in understanding the variations in diploid and fundamental numbers observed in *M. pusillus a*nd *P.pipistrellus.* In the latter species, there should be a need for further taxonomic studies in order to clarify its specific status.
- To reveal detailed genomic homology relationships and chromosomal changes among acrocentric or telocentric chromosomes of *p. pipistrellus* with heterochromatic regions, high resolution banding and other molecular techniques should be applied in future studies.
- Further chromosome study from other parts of the distribution range of bats is needed to document all the karyotypic forms.

- An integrated approach of all available data from karyological molecular and evolutionary studies is required to infer phylogeny relationships and to solve systematic problems.

Bats are probably the most seriously threatened group of mammals (Yalden and Morris, 1975). They are threatened at every stage of their life history: their insect food is diminishing and contaminated by insecticides; their breeding roosts suffer unwarranted disturbance and their specialized hibernation sites are rapidly disappearing. Therefore, bat conservation has to become a public issue and concerned governmental organizations and the public at large should protect them from extinction.

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APPENDICES

Appendix 1. Species of bats, site of capture, number of males and females and number of cells analyzed.

Species/specimen	Site captured	M	F	No. of cells analyzed
Chaerephon pumila	• Arba Minch Prepatatory School	3	2	30
	• Nechsar National Park	2	3	20
	• Ziway Kobo	1	1	20
Micropteropus pusillus	• Arba Minch Textile	2	3	20
	• Koka Tannery	2	1	42
	• Ziway Antonio Residence	2	2	40
	• Ziway Fruit Corporation Store	2	2	32
	• Ziway Kobo Banana Plantation	1		10
Pipistrellus pipistrellus	• Ziway Theology College	2	2	44
Scotophilus dinganii/viridis	• Ziway-Bridge near Kobo	1		20
DD5	• Kum Amba village near Alem Ketema (Merehabete region)	2	2	48
	Total	20	18	326

Appendix 2. The total length of each chromosome (µm), the arm lengths (µm) and the arm ratios of the chromosomes of ZA_1 (*Micropterpus pusillus* from Ziway Antonio Residence).

Mag: 2200
Image resolution: 59.06 pixels per cm

Marking order	Rank	Length each	Long arm	Short arm	Arm ratio
13	1	5.81	3.06	2.75	1.11
31	2	5.36	2.77	2.59	1.07
11	3	5.19	2.68	2.51	1.07
34	4	4.93	3.11	1.82	1.71
28	5	4.79	2.79	2.00	1.39
3	6	4.75	2.58	2.17	1.19
5	7	4.58	2.37	2.21	1.07
29	8	4.45	2.65	1.80	1.47
10	9	4.16	2.62	1.54	1.70
7	10	3.96	2.20	1.76	1.25
18	11	3.76	2.71	1.05	2.58
4	12	3.73	2.49	1.24	2.01
15	13	3.60	1.83	1.77	1.03
17	14	3.57	2.36	1.22	1.93
35	15	3.51	2.42	1.09	2.22
25	16	3.42	1.72	1.69	1.02
6	17	3.41	2.14	1.27	1.69
8	18	3.28	1.91	1.37	1.39
2	19	3.17	1.71	1.46	1.17
22	20	3.15	1.99	1.15	1.73
27	21	3.01	1.53	1.48	1.03
1	22	2.95	1.58	1.37	1.15
20	23	2.94	1.47	1.47	1.00
19	24	2.83	1.58	1.25	1.26
21	25	2.82	1.58	1.24	1.27
12	26	2.69	1.45	1.24	1.17
26	27	2.62	1.62	1.00	1.62
16	28	2.48	1.39	1.09	1.28
9	29	2.39	1.39	1.00	1.39
32	30	2.36	1.33	1.03	1.29
33	31	1.99	1.12	0.88	1.27
14	32	1.93	1.10	0.83	1.33
23	33	1.91	0.97	0.94	1.03
30	34	1.89	1.01	0.88	1.15
24	35	1.73	0.96	0.76	1.26

Appendix 3. The total length of each chromosome (µm), the arm lengths (µm) and the arm ratios of chromosomes of ZA_2 (*Micropteropus pusillus* from Ziway Antonio Residence).

Mag: 2200
Image resolution: 78.74 Pixels per cm

Marking order	Rank	Length each	Long arm	Short arm	Arm ratio
25	1	6.77	3.44	3.33	1.03
24	2	6.74	4.59	2.15	2.13
6	3	6.54	3.42	3.12	1.09
15	4	6.51	3.99	2.52	1.58
7	5	6.41	3.35	3.06	1.09
30	6	6.33	3.60	2.72	1.32
33	7	6.02	3.35	2.67	1.25
32	8	5.78	3.24	2.54	1.28
16	9	5.04	3.23	1.81	1.78
9	10	4.81	3.41	1.39	2.45
3	11	4.56	2.62	1.94	1.35
10	12	4.53	3.11	1.43	2.17
14	13	4.46	3.07	1.39	2.21
21	14	4.39	3.10	1.29	2.40
17	15	4.35	2.67	1.68	1.59
27	16	4.33	2.86	1.47	1.95
2	17	4.28	2.64	1.63	1.62
23	18	4.26	2.46	1.80	1.37
13	19	4.21	2.13	2.08	1.02
35	20	4.10	2.09	2.00	1.05
1	21	4.07	2.71	1.36	1.99
8	22	3.99	2.20	1.79	1.23
11	23	3.93	2.05	1.88	1.09
29	24	3.88	2.14	1.74	1.23
5	25	3.79	2.02	1.78	1.13
22	26	3.58	2.09	1.49	1.40
18	27	3.49	1.91	1.59	1.20
12	28	2.99	1.61	1.39	1.16
36	29	2.83	1.68	1.16	1.45
28	30	2.72	1.65	1.06	1.56
26	31	2.39	1.28	1.11	1.15
31	32	2.39	1.22	1.17	1.04
4	33	2.31	1.39	0.91	1.53
34	34	2.08	1.16	0.93	2.25
19	35	1.87	1.11	0.76	1.46
20	36	1.81	1.10	0.70	1.57

Appendix 4. The total length of each chromosome (μm), the arm lengths (μm) and the arm ratios of the chromosomes of ZF_1 (*Micropteropus pusillus* from Zeway Fruit Store).

Mag: 2200
Image resolution: 59.06 pixels per cm

Marking order	Rank	Length each	Long arm	Short arm	Arm ratio
3	1	6.20	3.89	2.31	1.68
1	2	5.70	2.92	2.78	1.05
15	3	5.68	3.06	2.62	1.17
31	4	5.59	2.80	2.79	1.00
10	5	5.43	2.77	2.66	1.04
26	6	5.31	3.17	2.14	1.48
29	7	5.25	3.65	1.60	2.28
27	8	5.04	2.78	2.26	1.23
25	9	4.46	2.66	1.79	1.49
18	10	4.32	3.01	1.31	2.29
23	11	4.23	2.56	1.66	1.54
13	12	4.17	2.46	1.70	1.45
17	13	4.06	2.52	1.54	1.64
9	14	4.01	2.17	1.85	1.17
7	15	3.94	2.19	1.76	1.24
6	16	3.87	2.32	1.56	1.49
21	17	3.84	2.08	1.76	1.18
5	18	3.63	2.42	1.22	1.98
12	19	3.56	2.48	1.08	2.29
8	20	3.54	1.97	1.57	1.25
22	21	3.51	1.76	1.76	1.00
32	22	3.28	2.29	0.98	2.34
19	23	3.25	1.69	1.56	1.08
24	24	3.17	1.78	1.39	1.28
11	25	2.90	1.56	1.35	1.16
2	26	2.85	1.64	1.22	1.34
16	27	2.69	1.60	1.09	1.47
30	28	2.63	1.32	1.31	1.01
28	29	2.56	1.56	1.00	1.56
33	30	2.51	1.27	1.24	1.02
20	31	2.27	1.23	1.03	1.19
4	32	2.19	1.19	1.00	1.19
34	33	1.94	1.08	0.86	1.23
14	34	1.83	1.10	0.73	1.51
35	35	1.51	0.85	0.66	1.29

Appendix 5. The total length of each chromosome (µm), the arm lengths (µm) and the arm ratios of the chromosomes of KT_1 (*Micropteropus pusillus* from Koka Tannery).

Mag: 2200
Image resolution: 59.06 Pixels per cm

Marking order	Rank	Length each	Long arm	Short arm	Arm ratio
20	1	5.57	3.01	2.56	1.18
32	2	5.49	3.34	2.16	1.55
7	3	5.45	3.24	2.21	1.47
30	4	5.37	2.79	2.58	1.08
8	5	5.29	2.82	2.48	1.14
15	6	4.82	2.82	2.00	1.41
1	7	4.80	2.41	2.39	1.01
5	8	4.24	2.47	1.78	1.39
27	9	4.11	2.86	1.24	2.31
25	10	3.88	2.01	1.86	1.08
24	11	3.84	2.18	1.66	1.31
29	12	3.79	2.34	1.45	1.61
22	13	3.69	2.22	1.48	1.50
3	14	3.69	2.10	1.59	1.32
2	15	3.67	2.51	1.15	2.18
10	16	3.65	2.34	1.31	1.79
13	17	3.50	2.16	1.34	1.61
14	18	3.49	1.99	1.49	1.34
9	19	3.47	1.93	1.54	1.25
19	20	3.45	1.90	1.55	1.23
16	21	3.42	1.76	1.66	1.06
6	22	3.36	2.46	0.89	2.76
21	23	3.12	2.32	0.80	2.90
35	24	3.03	1.74	1.28	1.36
33	25	2.89	1.58	1.31	1.21
23	26	2.83	1.46	1.36	1.07
12	27	2.62	1.46	1.15	1.27
4	28	2.52	1.41	1.11	1.27
11	29	2.38	1.23	1.14	1.08
18	30	2.38	1.34	1.03	1.30
17	31	2.15	1.15	1.00	1.15
34	32	2.14	1.15	0.99	1.16
28	33	2.13	1.35	0.78	1.73
31	34	2.03	1.15	0.88	1.31
26	35	1.92	1.07	0.85	1.26

Appendix 6. The total length of each chromosome (µm), the arm lengths (µm) and the arm ratios of the chromosomes of KT_2 (*Micropteropus pusillus* from Koka Tannery)

Mag: 2200
Image resolution: 78.74 Pixels per cm

Marking order	Rank	Length each	Long arm	Short arm	Arm ratio
28	1	4.23	2.42	1.81	1.34
2	2	4.13	2.29	1.85	1.24
34	3	4.12	2.19	1.92	1.14
31	4	4.11	2.25	1.86	1.21
35	5	4.09	2.34	1.76	1.33
5	6	4.00	2.81	1.19	2.36
13	7	3.43	1.73	1.69	1.02
9	8	3.38	1.78	1.59	1.12
30	9	2.92	1.57	1.35	1.16
32	10	2.89	1.73	1.16	1.49
21	11	2.88	1.71	1.17	1.46
1	12	2.80	1.99	0.81	2.46
20	13	2.75	1.84	0.90	2.04
27	14	2.73	1.81	0.92	1.97
25	15	2.69	1.48	1.22	1.21
3	16	2.69	1.76	0.94	1.87
16	17	2.67	1.43	1.23	1.16
6	18	2.61	1.86	0.75	2.48
3	19	2.59	1.33	1.27	1.05
24	20	2.58	1.51	1.06	1.42
12	21	2.49	1.28	1.22	1.05
10	22	2.42	1.23	1.19	1.03
26	23	2.41	1.42	0.99	1.43
4	24	2.21	1.11	1.09	1.02
14	25	2.18	1.22	0.96	1.27
7	26	2.15	1.17	0.98	1.19
19	27	2.14	1.22	0.91	1.34
11	28	1.80	1.17	0.64	1.83
8	29	1.72	0.97	0.75	1.29
17	30	1.70	1.03	0.67	1.54
29	31	1.67	0.90	0.77	1.17
18	32	1.66	0.97	0.69	1.41
15	33	1.51	0.81	0.70	1.16
22	34	1.45	0.87	0.58	1.50
33	35	1.35	0.72	0.62	1.16

Appendix 7. The total length of each chromosome (μm), the arm lengths (μm) and the arm ratios of the chromosomes of ZC_3 (*Pipistrellus pipistrellus* from Ziway Theology College).

Mag:	2200	
Image resolution:	78.74	Pixels per cm

Marking order	Rank	Length each	Long arm	Short arm	Arm ratio
21	1	6.78	3.59	3.19	1.13
29	2	6.54	3.29	3.25	1.01
34	3	6.08	3.25	2.83	1.15
4	4	5.75	2.89	2.87	1.01
33	5	5.65	3.09	2.56	1.21
22	6	5.55	3.07	2.48	1.24
12	7	5.29	3.12	2.17	1.44
1	8	5.19	2.66	2.52	1.06
28	9	5.16	2.66	2.50	1.06
20	10	5.11	2.59	2.52	1.03
3	11	4.56	2.36	2.20	1.07
8	12	4.52	2.42	2.09	1.16
2	13	4.51	2.32	2.19	1.06
14	14	4.39	2.43	1.96	1.24
9	15	3.62	N/A	N/A	
19	16	3.52	N/A	N/A	
25	17	2.96	N/A	N/A	
24	18	2.66	N/A	N/A	
10	19	2.57	1.59	0.98	1.62
32	20	2.46	1.50	0.96	1.56
31	21	2.39	N/A	N/A	
16	22	2.35	N/A	N/A	
11	23	2.25	N/A	N/A	
30	24	2.07	N/A	N/A	
15	25	2.06	N/A	N/A	
6	26	2.03	N/A	N/A	
5	27	1.99	1.21	0.79	1.53
35	28	1.99	N/A	N/A	
26	29	1.92	N/A	N/A	
7	30	1.92	N/A	N/A	
18	31	1.84	N/A	N/A	
17	32	1.64	N/A	N/A	
36	33	1.57	N/A	N/A	
27	34	1.48	N/A	N/A	
13	35	1.33	N/A	N/A	
23	36	1.21	N/A	N/A	

Appendix 8. The total length of each chromosome (µm), the arm lengths (µm) and the arm ratios of the chromosomes of ZB_2 (*Scotophilus dinganii (S. viridis)* from Ziway bridge near Kobo).

Mag:	2200	
Image resolution:	59.06	Pixels per cm

Marking order	Rank	Length each	Long arm	Short arm	Arm ratio
11	1	4.72	3.09	1.62	1.91
29	2	4.56	2.65	1.91	1.39
32	3	4.55	2.63	1.92	1.37
1	4	4.49	2.67	1.83	1.46
33	5	4.11	2.07	2.03	1.02
31	6	3.90	2.37	1.53	1.55
23	7	3.57	1.98	1.59	1.25
13	8	3.51	3.02	0.49	6.16
22	9	3.33	2.95	0.38	7.76
25	10	3.29	1.69	1.61	1.05
4	11	3.22	2.29	0.94	2.44
27	12	3.22	2.56	0.66	3.88
35	13	3.17	1.92	1.24	1.55
6	14	3.15	1.64	1.52	1.08
34	15	3.15	1.69	1.45	1.17
19	16	3.15	2.80	0.34	8.24
21	17	3.09	N/A	N/A	
3	18	3.04	2.72	0.32	8.50
10	19	3.04	1.97	1.07	1.84
16	20	3.00	N/A	N/A	
30	21	3.00	1.99	1.00	1.99
12	22	2.99	N/A	N/A	
17	23	2.95	2.63	0.32	8.22
20	24	2.92	2.38	0.54	4.41
7	25	2.92	1.77	1.14	1.55
2	26	2.86	1.70	1.16	1.47
9	27	2.69	2.47	0.23	10.74
28	28	2.66	2.34	0.32	7.31
8	29	2.64	1.69	0.95	1.78
24	30	2.27	1.16	1.11	1.05
15	31	2.25	1.49	0.76	1.96
18	32	2.13	1.90	0.23	8.26
14	33	2.10	1.18	0.93	1.27
26	34	2.09	1.47	0.62	2.37
5	35	1.93	1.09	0.83	1.31
36	36	1.31	N/A	N/A	

Appendix 9. The total lengths of each chromosome (µm), the armlength of (µm) and the arm ratios of the chromosomes of DD5 (*Specimen* from Dim-Dim cave of Kum-Amba near Alem Ketema).

Mag: 2200
Image resolution: 59.06 Pixels per cm

Marking order	Rank	Length each	Long arm	Short arm
36	1	6.10	3.88	2.22
6	2	5.56	3.06	2.49
22	3	5.37	2.75	2.61
38	4	5.27	2.65	2.62
13	5	5.18	2.72	2.46
37	6	4.83	2.82	2.
23	7	4.80	2.54	2.26
30	8	4.79	2.68	2.12
21	9	4.78	2.63	2.16
41	10	4.72	2.75	1.97
31	11	4.72	3.01	1.71
14	12	4.69	2.39	2.31
8	13	4.62	2.46	2.15
15	14	4.61	2.51	2.10
42	15	4.59	2.29	2.29
39	16	4.55	2.39	2.16
18	17	4.51	2.61	1.90
1	18	4.46	2.43	2.03
33	19	4.20	2.48	1.72
35	20	4.14	2.36	1.78
20	21	4.11	2.21	1.90
32	22	4.03	2.07	1.96
24	23	3.98	2.17	1.82
9	24	3.97	2.00	1.97
12	25	3.94	2.29	1.65
7	26	3.86	2.00	1.86
19	27	3.85	2.36	1.49
17	28	3.75	1.93	1.82
40	29	3.55	1.96	1.59
4	30	3.53	2.38	1.16
26	31	3.50	1.95	1.56
34	32	3.44	1.81	1.62
16	33	3.42	2.02	1.40
2	34	3.31	1.77	1.53
10	35	3.27	1.85	1.41

27	36	3.18	1.68	1.49
29	37	3.16	1.69	1.47
28	38	2.92	1.74	1.17
3	39	2.86	1.47	1.39
5	40	2.78	1.47	1.31
25	41	2.48	N/A	N/A
11	42	1.82	N/A	N/A