Karyotype Description of bats from different localities in Ethiopia

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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.

Index Terms- Bat, 2n number, Fundamental number, Karyotype, Ethiopia, Chromosome.

I. INTRODUCTION

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.

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).

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.

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

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	06054'N 40048'E	1340m	11
4	Koka	$08^0$26'N $39^0$01'E	1700m	9
5	Lake Abiata	07037'N 38039'E	1590m	13
6	Arba Minch	$05^0$57'N $37^0$32'E	1400m	11
7	Bulcha Forest	06011'N 38010'E	1800m	9
8	Didessa River	$09^0$02'N $36^0$09'E	1200m	15
9	Gambela	08015'N 34035'E	515m	27

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.Banding also permits the recognition of chromosome deletions, duplications and other types of structural rearrangements of chromosomes.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). The other definition includes only the arms of the autosomal chromosomes and is referred to as autosomal fundamental number (NFa).

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.

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.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.

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).

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.

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

2.1Collection of specimens

2.1.1.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.

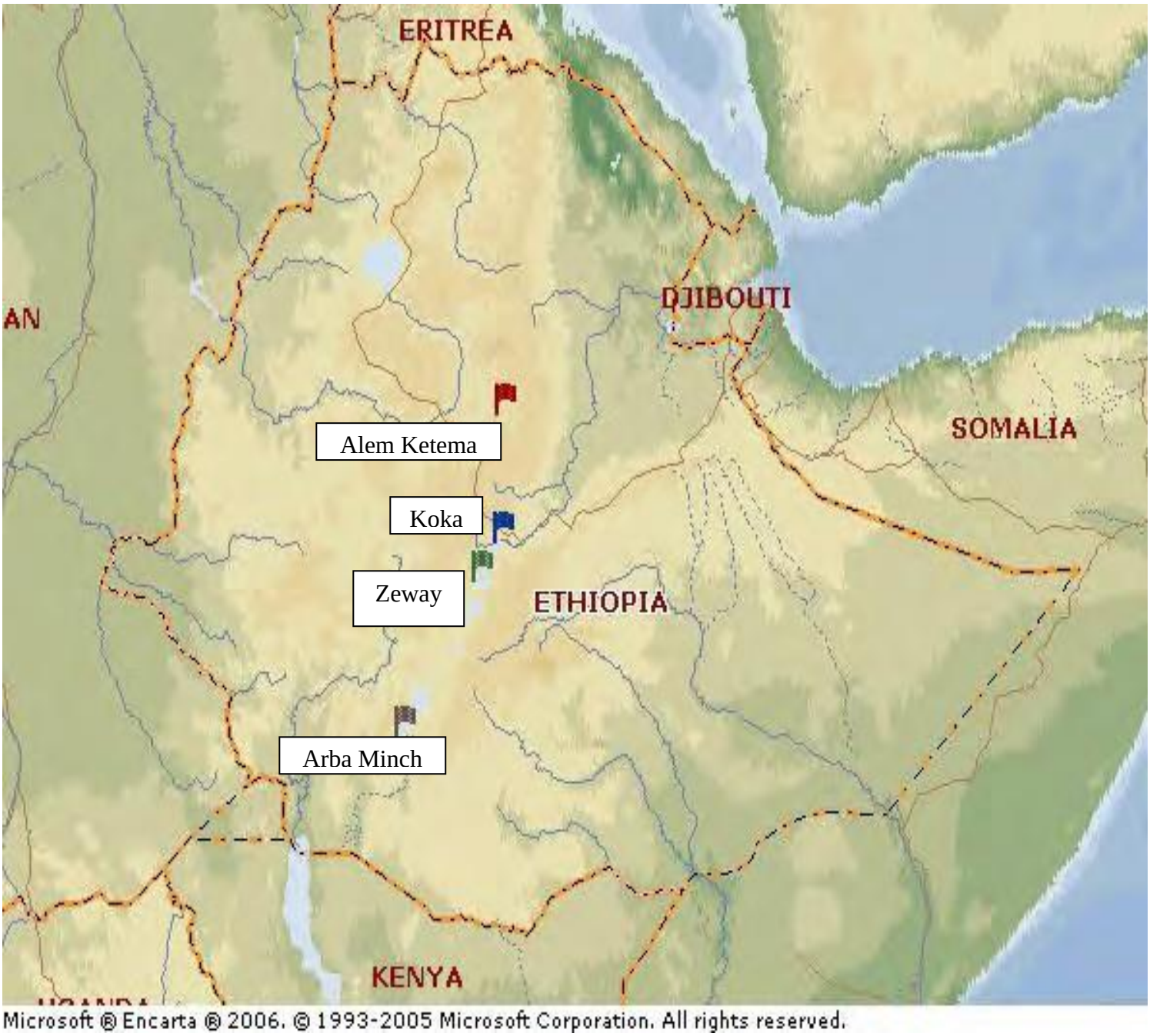
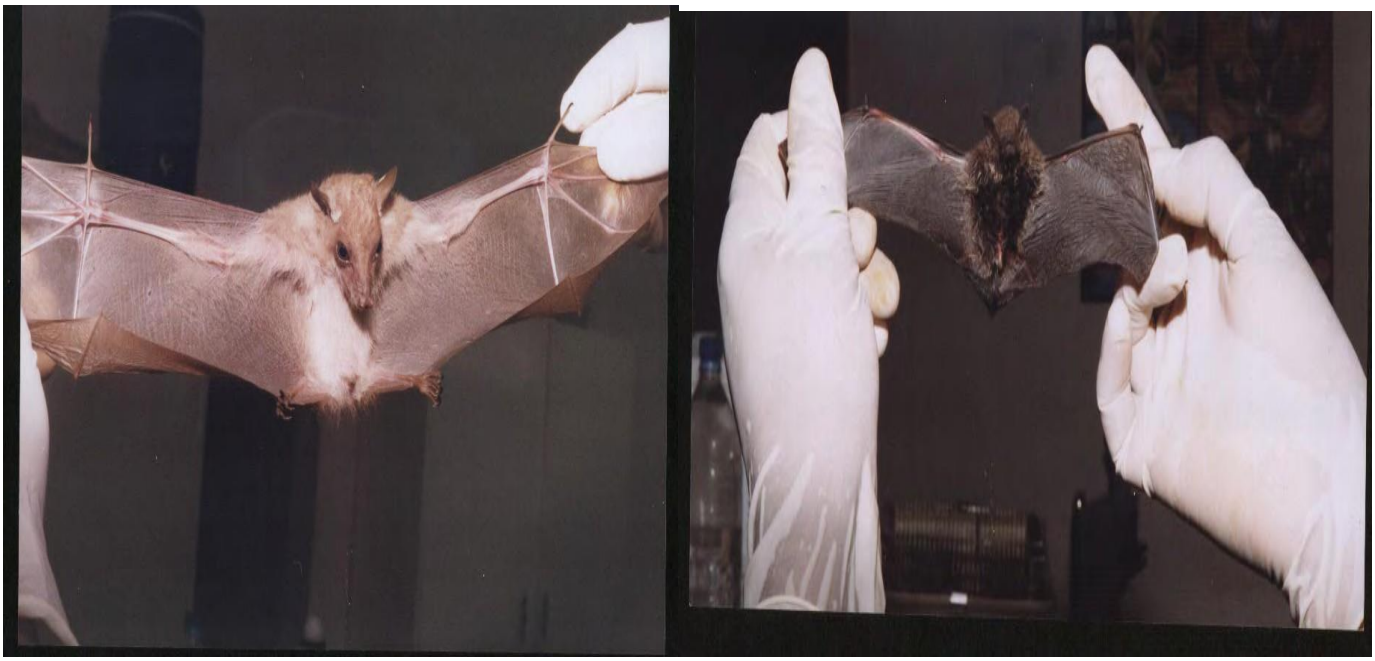


Table 3. The species of bats, geographic location and the number of bats captured.

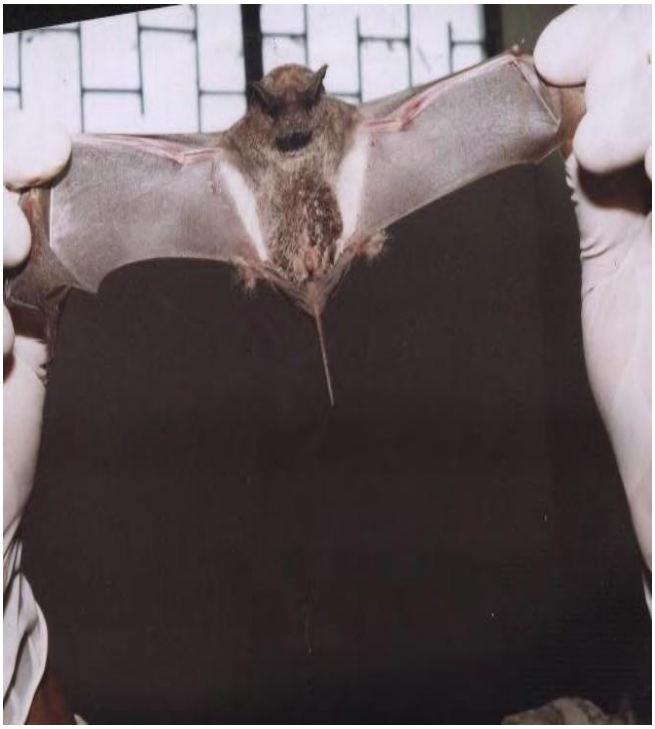
Species	Locality	Geographic location	Male	Female	Total
Chaerephon pumila	Ziway Arba Minch	$7^0$5'N, 38^0 42' E $5^0$57' N 37^0 32' E	1 5	1 5	2 10
Micropteropus pusillus	Ziway Koka Arba Minch	$7^0$5'N, 38^0 42' E 8^0 26' N 39^0 01'E $5^0$57' N 37^0 32' E	5 2 2	4 1 3	9 3 5
Pipistrellus pipistrellus	Ziway	705'N, 380 42' E	2	2	4
Scotophilus dinganii/viridis	Ziway	705'N, 380 42' E	1		1
DD5	Alem Ketema (Merehabete)	10^0 04' N 39^0 01'E	2	2	4
		Total	20	18	38

2.1.2 Method of Capturing

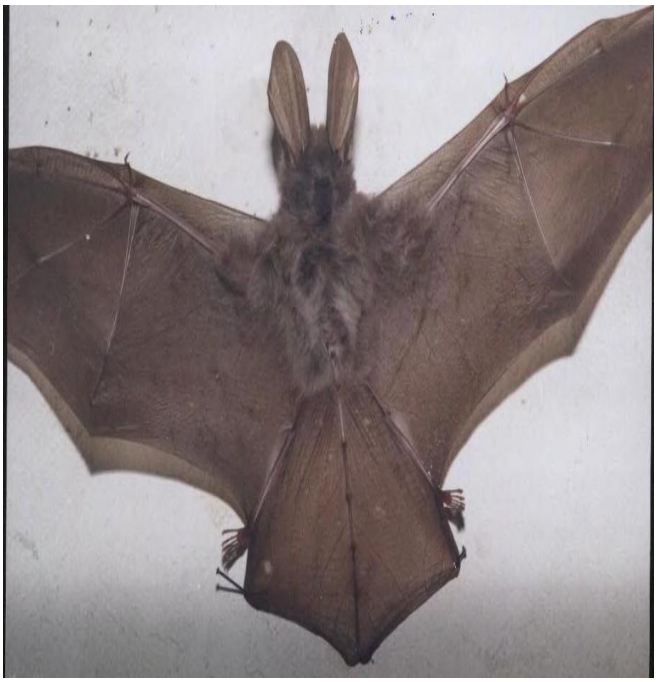
A large butterfly net was used at the exit of caves and roof of buildings in addition to large fishnets. Trapping with nets, spread out on frames or draped over escape holes was another method used to capture bats. Hand collecting method was also used in a banana plantation where the height of the banana 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

2.1.3. 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).

2.1.4. 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.

2.1.5. 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.

2.1.6. 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.

III. RESULT

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

3.1. Karyotype Description of *Micropteropus pusillus*

Somatic metaphase chromosome analysis has been made from 11 specimens of this species collected from Koka (KT1-KT3), Ziway(ZA1-ZA3,ZF1-ZF3 and ZKM) and Arba Minch(AT3).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).

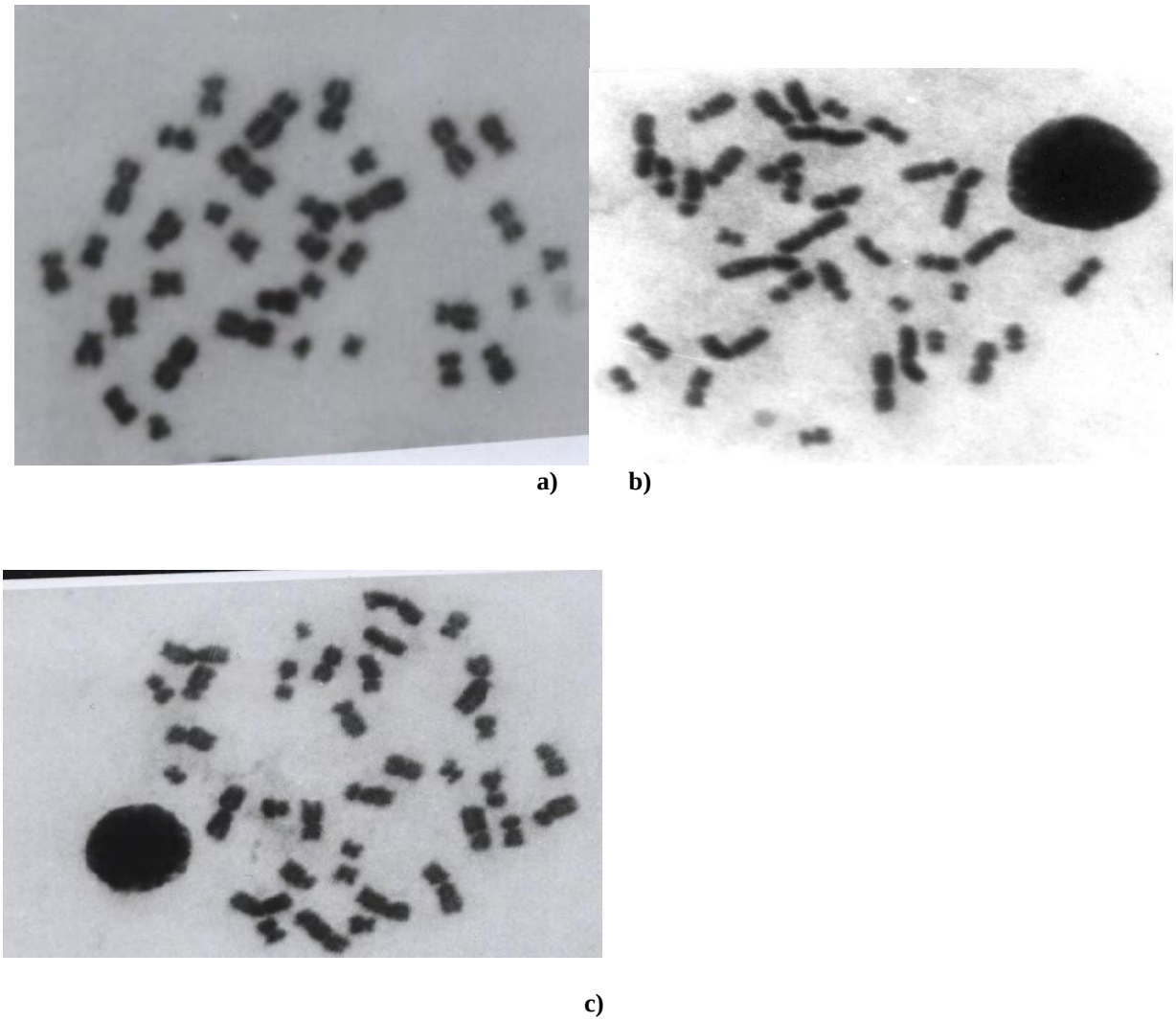
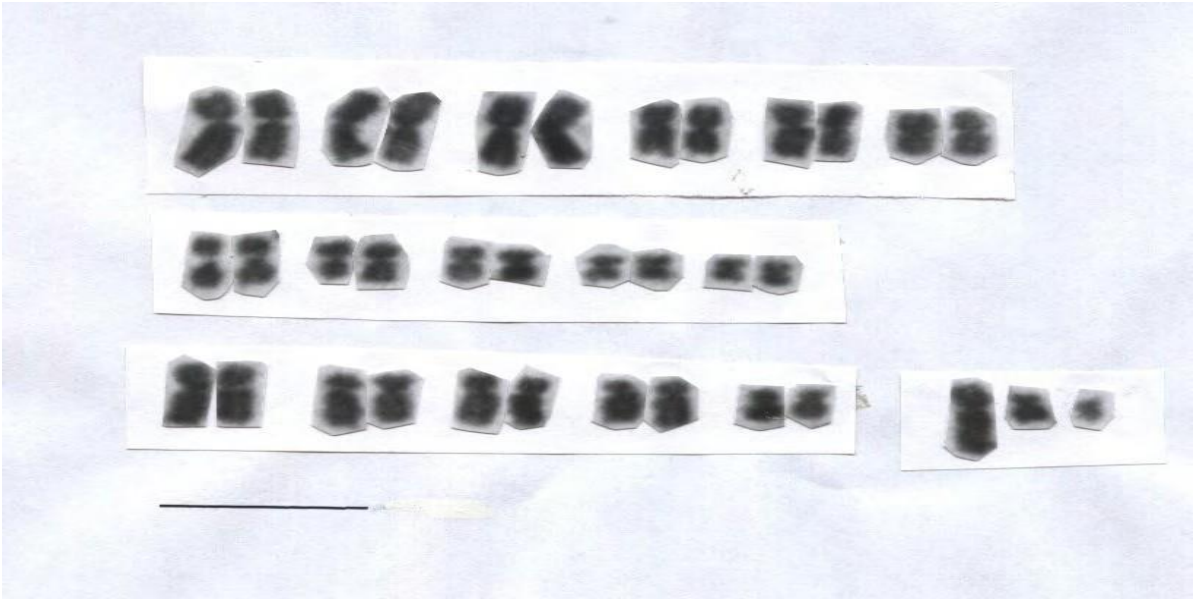
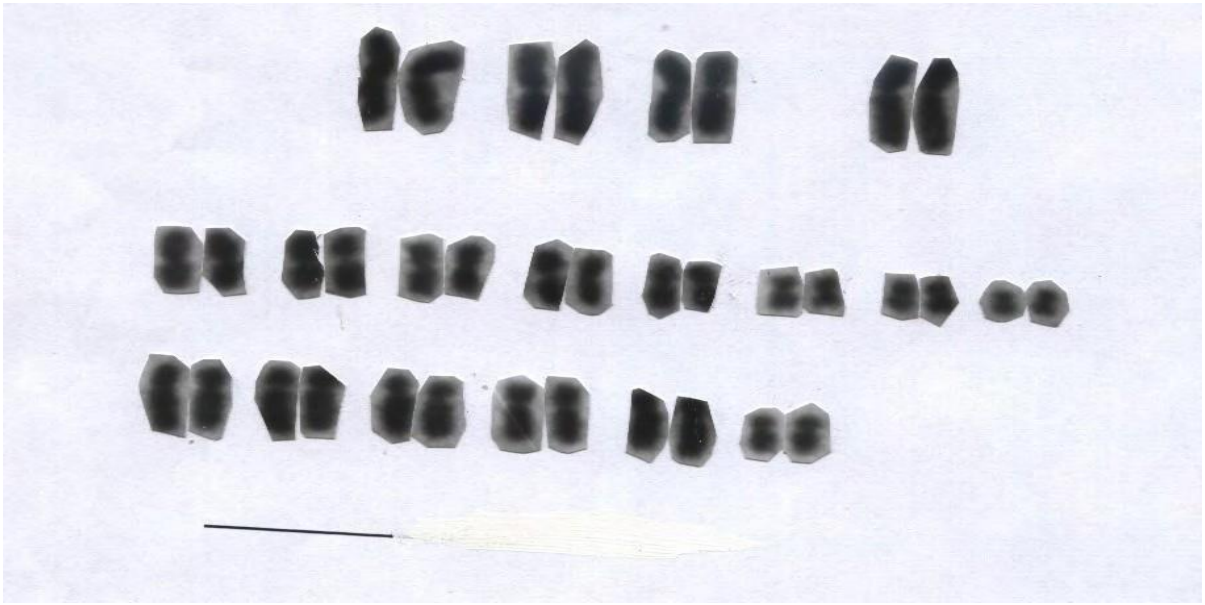


Fig.3. Mitotic metaphase chromosome spreads of *Micropteropus pusillus* specimens;
a) male from Ziway (2n=35); b) male from Ziway(2n=36); c) male from Koka (2n=36).



a)



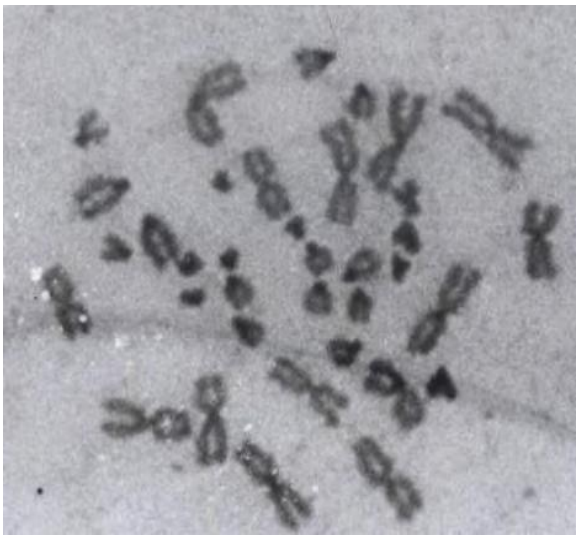
b)

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

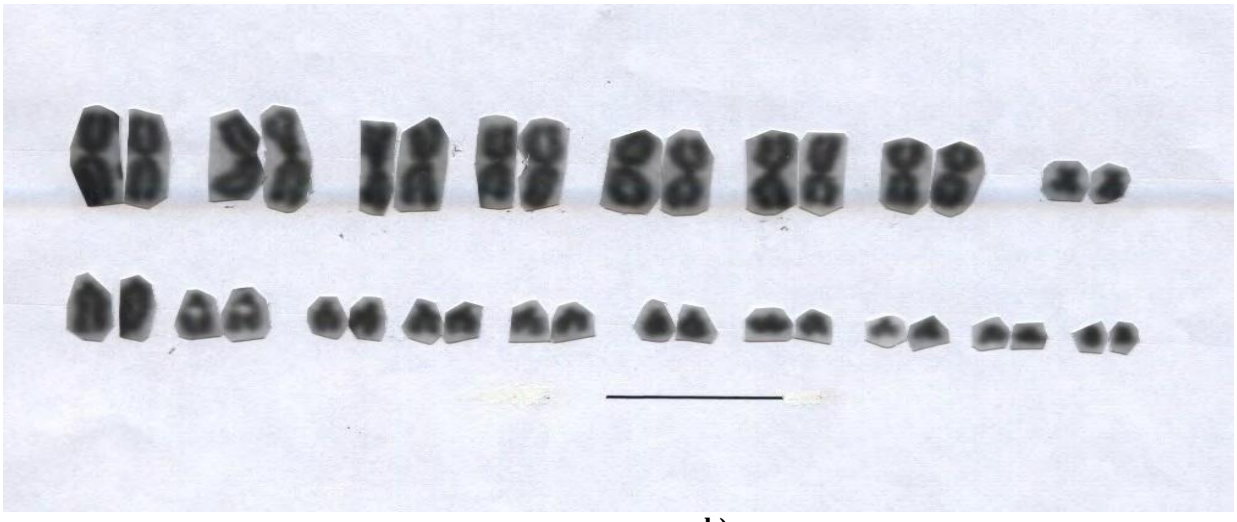
3.2. Karyotype Description of *Pipistrellus pipistrellus*

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)



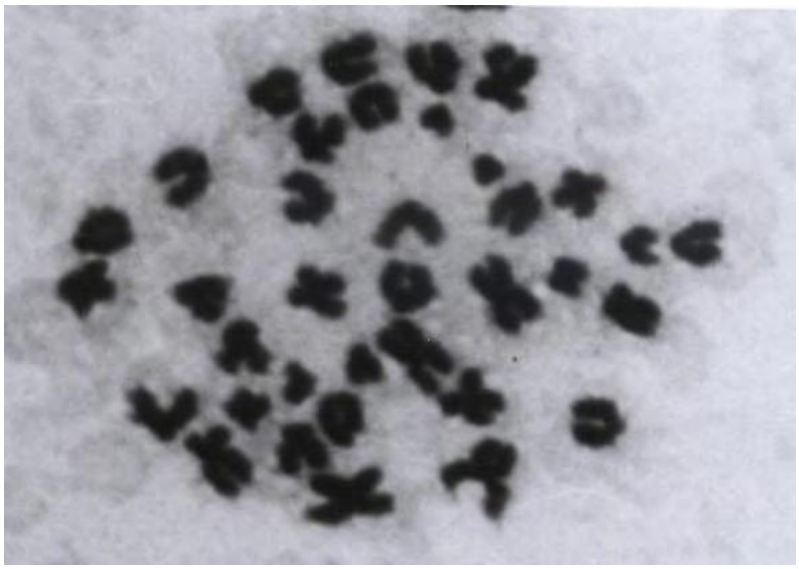
b)

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

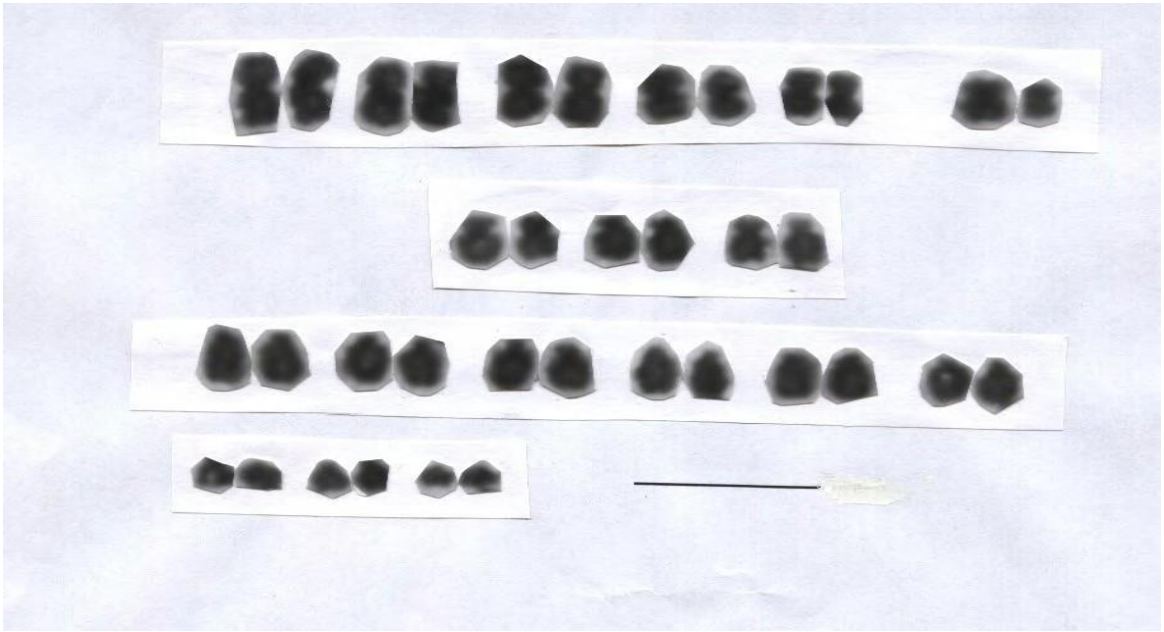
3.3. Karyotype Description of *Scotophilus* species

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

3.4. 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 (ZK1 and ZK2). 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) ZK1 (male) and b) ZK2 (Female)

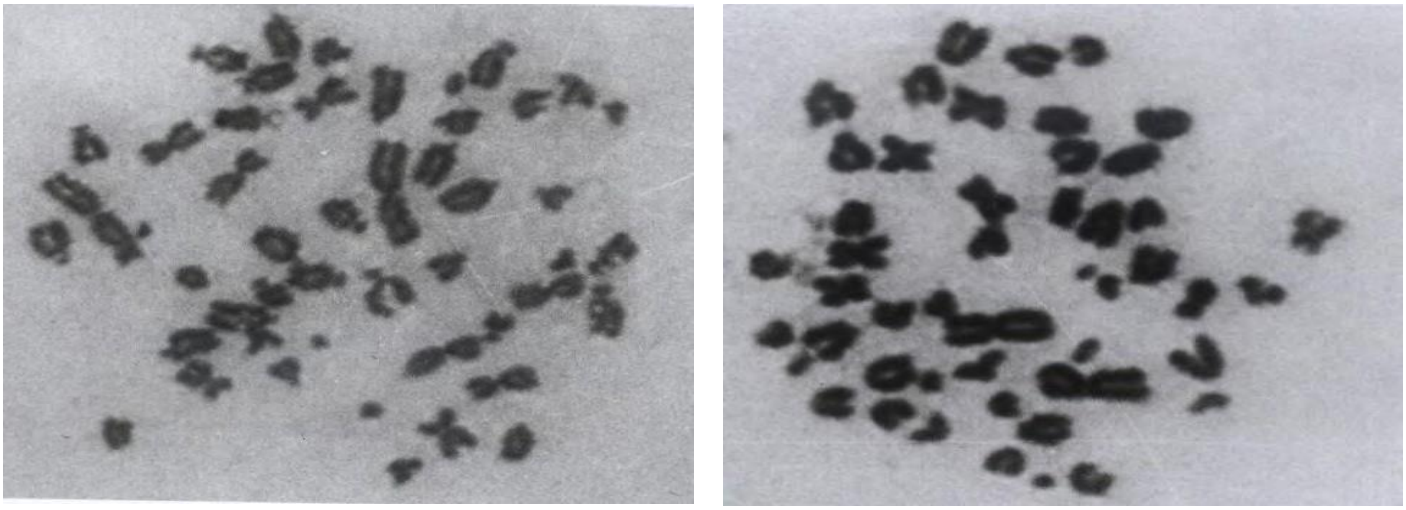
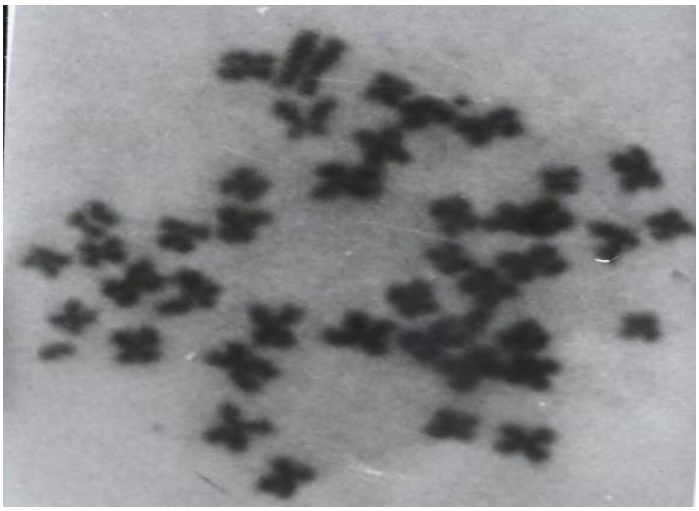


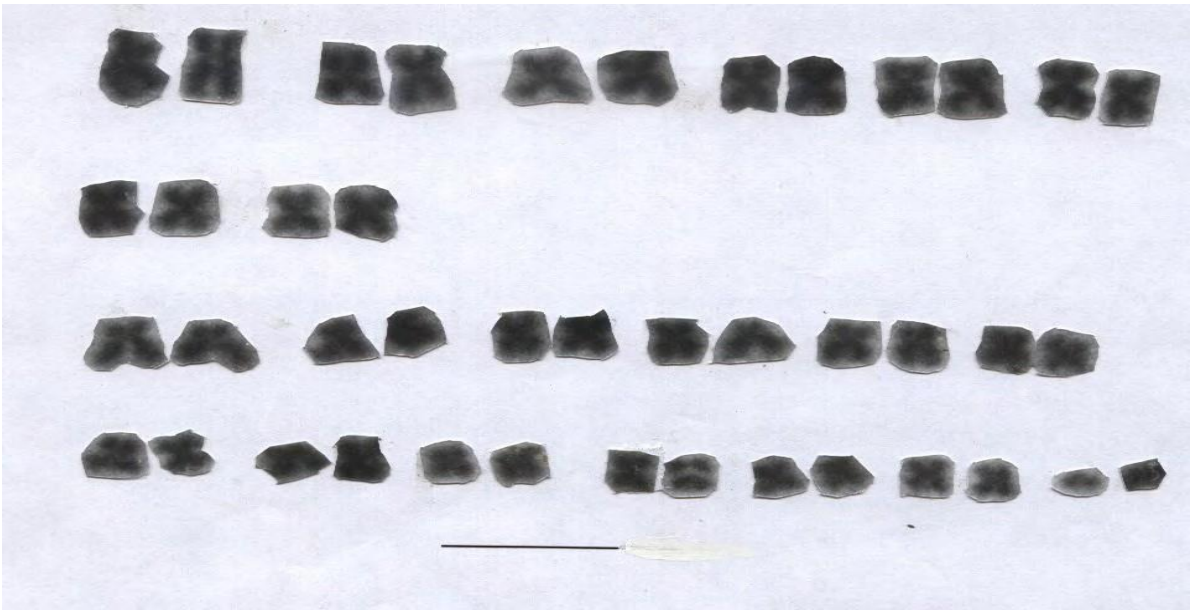
Fig. 8 Karyotype of male *Chaerephon pumila*. Bar = 2.4µm

3.5. Karyotype Description of Specimen from Merehabete region

A specimen given a code DD5 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).



a)



b)

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
ZA1		23	12				35	70
ZA_2		24	12				36	72
ZF_1		23	12				35	70
KT1		23	12				35	70
KT2		23	12				35	70
KT_2		24	12				36	72
ZC3		14	2			20	36	52
ZB2		12		3	3	18	36	56
DD5		36	4			2	42	82

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

IV. 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 (KT2, ZA2 and ZKM) and from Arba Minch (AT3) 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 XY1Y2 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 (DD5) 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.

V. 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.

VI. 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 ZA2 (*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 ZF1 (*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

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