# ADDIS ABABA UNIVERSITY

# RESEARCH AND GRADUATE PROGRAM OFFICE

# CHROMOSOME STUDY OF THE COMMON MOLE RAT (*Tachyoryctes splendens*) FROM DIFFERENT LOCALITIES IN ETHIOPIA

**By**

**ZIYIN MIHRETIE ASMARE**

**Addis Ababa, June 2005**

# Chromosome Study of the Common Mole Rat (*Tachyoryctes splendens*) from Different Localities in Ethiopia

**A Thesis submitted to the Research and Graduate Program Office of the Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Biology**

**(Applied Genetics)**

**By**

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ABSTRACT

The common mole rat, *Tachyoryctes splendens,* belongs to family Rhizomyidae or Muridae (Mammalia; Rodentia). Somatic chromosomes of the species were studied from 9 localities in Ethiopia (Mugo, Masha, Bure, Ziway, Asebe Teferi, Alemaya, Entoto, Sebeta and Debre Sina) using the standard Giemsa staining and C-banding techniques. Six different karyotypic forms were found. These variations were revealed as differences in chromosome morphology, autosomal fundamental number, amount and distribution of heterochromatin. However, there was no variation in diploid chromosome number and specimens from all the localities had 2n=48. The six karyotypic forms had the following karyotypic formulae: Mugo, Masha and Bure (12m + 10st + 26a), Debre Sina (10m + 8st + 30a), Ziway (8m + 12st + 28a), Asebe Teferi (8m + 4st + 36a), Alemaya (8m + 6st + 34a), and Entoto and Sebeta (7m + 1 sm + 2st + 38a). C-banding revealed that, in addition to the Y-chromosome (except in Entoto), 12, 12, 36, 4, 6, 4, 4 autosomes in Mugo, Masha, Ziway, Asebe Teferi, Alemaya, Entoto and Sebeta were C-positives, respectively. The telomeric region of the long arm of the X-chromosome was also C-positive in animals from Entoto and Sebeta. The present study did not confirm the earlier suggestions that the number of biarmed chromosomes of *T. splendens* decreases with the increase in aridity and the number of heterochromatic arms may exhibit an altitude gradient. We recommend further studies like G-banding, zoogeographical, physiological and hybridization studies on *T. splendens* from different parts of the distribution range of the species so that the results could supply information to reveal factors associated with the existing variations, and to draw a valid conclusion that could be used in clearing the taxonomic uncertainties in this animal.

**Key words/ Phrases**: *Tachyoryctes splendens,* chromosome, karyotype, C-banding, Ethiopia

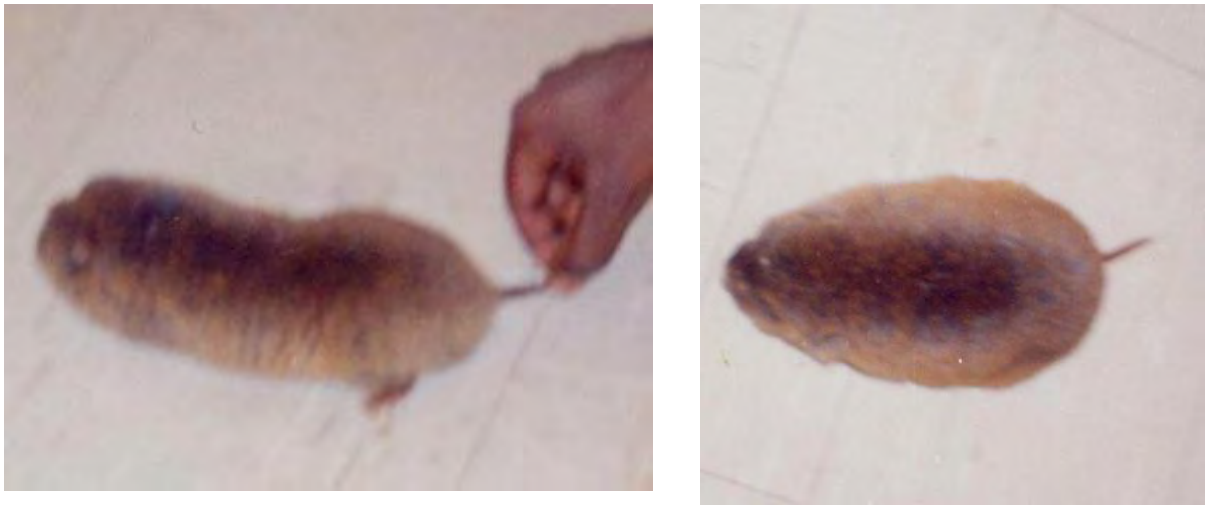
# 1. INTRODUCTION

## 1.1. Taxonomy

The common mole rat, *Tachyoryctes splendens*, belongs to the genus *Tachyoryctes* Ruppell 1835 of the mammalian order Rodentia. Though the exact number of species belonging to this genus is not clearly known (Nowak, 1991), a varying number of species have been reported. Some authors (Anderson and Jones, 1967; Sokolov, 1977; Honacki *et al*., 1982; Corbet and Hill, 1991) consider the number of species of *Tachyoryctes* to be two by lumping all known forms, except *T. macrocephalus*, into *T. splendens*. On the other hand, Ellerman (1941) considers *T. splendens* to be only one of the 14 species of this genus. Furthermore, this genus has no fixed taxonomic position. Ellerman (1941) placed it in the family Muridae, while Allen (1939) cited by Baskevich *et al*. (1993), Anderson and Jones (1967), Sokolov (1977), Honacki *et al*. (1982) placed the genus in the family Rhizomyidae, which includes two other genera namely, *Rhyzomys,* Gray 1831 and *Cannomys,* Thomas 1915. The common mole rat, *T. splendens*, is locally named as *filfel* (Amharic), *tuka* (Oromifa).

## 1.2. Description of *T. splendens*

The external form of *T. splendens* is basically rat-like (Fig. 1.). According to Jarvis and Sale (1971), the adult males weigh an average of 250 g with an average head and body length of 202 mm. Females average 218 g with an average head and body length of 194 mm. Nowak (1991) described the animal as follows: the short tail is about twice the length of the hind foot and usually well haired; the fur is thick and soft; coloration is variable-some are shining black, while others are brownish, reddish brown, pale gray, or cinnamon buff; most young are black; albinos and partial albinos are as common as black adults; the under parts are usually slightly paler than the upper parts; small, plainly visible and functional eyes; small ears; stiff hairs on the face; the thick, projecting incisors are not grooved and are deep orange in color; although the claws are not particularly large, the hands and feet are well developed, and the short, powerful legs are suited for digging.



A B

Fig. 1. The common mole rat, *T. splendens*, from Sebeta: plate (A) seen from the side and plate (B) seen from the top.

### 1.3. Distribution and Habitat of *T. splendens*

*Tachyoryctes splendens* is a solitary, aggressive, fossorial rodent, which is distributed over the uplands of north eastern Africa, from Ethiopia to as far as eastern Zaire, including parts of Somalia, Kenya, Rwanda, Burundi, Uganda and northern Tanzania (Jarvis and Sale, 1971; Nowak, 1991). The species is usually found in areas with more than 500 mm of annual rainfall and flourish best in wet uplands. It favors open grassland, thinly treed savannah and cultivated areas. In Ethiopia, the species is mostly found between 1300 and 3900 meters above see level (Yalden *et al*., 1976 cited in Sewnet Mengistu and Afework Bekele, 2003). It can also live at elevation of upto 4,150 meters above sea level (Kingdon, 1974; Nowak, 1991)

Although *T. splendens* is the least modified for fossorial life of the East African mole rats, it can construct a burrow system consisting of a nest chamber, a bolt-hole, and a number of foraging tunnels. The nest chamber, is used for sleeping, storing food and sanitation. The bolt-hole is used as place of retreat. The foraging burrow usually run just below the grass root level and its length is determined by the availability of food. The total length of a burrow system may reach upto 52 meters. When the burrow is excavated, mounds of earth about 15-40cm wide and 7-15cm high are formed (Jarvis and Sale, 1971; Kingdon, 1974; Nowak, 1991). Jarvis and Sale (1971)

observed, using captive animals, that all burrow digging is done with the incisors except where the soil is very soft. Where the soil is very soft and loosened, the animals use their forefeet for scratching the soil up.

### 1.4. Feeding

*Tachyoryctes splendens* feeds mainly on the underground parts of plants: roots, rhizomes, tubers, bulbs, and corms. It sometimes comes to the surface to forage for nesting materials and food, such as grasses and cultivated legumes (Jarvis and Sale, 1971; Kingdon, 1974; Nowak, 1991). The animal gets fresh food supply when it needs since the foraging burrow is usually just below the root level. When foraging, the mole rat takes what it needs from the exposed parts of plants in the burrow (Jarvis and Sale, 1971). It also stores food in the nest chamber. Such a feeding behavior of *T. splendens* makes it an agricultural pest. It causes a great damage to crops like enset (*Ensete ventricosum*), potato (*Solanum tuberosum*), and maize (*Zea mays*) in southern, western and central regions of Ethiopia (Sewnet Mengistu and Afework Bekele, 2003).

### 1.5. Reproduction

Although the breeding activity of *T. splendens* is highest during the rains and lowest in the dry season, it continues throughout the year (Nowak, 1991). Jarvis (1973) found an average annual litter size of 2.1 per female and gestation period of 37-40 days in Kenya, while Rham (1969) determined the gestation period to be 45-50 days in eastern Zaire (Congo). Females may deliver up to four young per litter, but usually only one or two. The young are weaned at 4-6 weeks, leave the mother about one month later, reach sexual maturity at 6 months of age, and the average life expectancy is about 1 year (Nowak, 1991). The species is solitary, and each adult lives alone in its own burrow system.

# 2. LITERATURE REVIEW

## 2.1. Chromosome Study

Studying chromosome number and karyotype has generally been useful for taxonomic purposes and for possible clues to the evolution of related groups of species (Garber, 1979). Each chromosome has unique topological features and shows different banding patterns when treated in various ways.

Subterranean mammals generally show chromosomal variations; this is because the low level of migration increases the pressure of isolation and the possibility of fixation of chromosome mutations (Aniskin *et al*. 1997).

Even though karyotype is generally a species characteristic (Fredga, 1977), there are a number of examples of intraspecific and even intrapopulational variability in mammals especially in the order Rodentia (Baskevich *et al*., 1993). The common shrew has, together with the house mouse, the most variable karyotype of all mammal species (Fredga, 2003). Such chromosomal variation is also the characteristics of *T. splendens* (Baskevich *et al*. 1993; Aniskin *et al*., 1997).

### 2.1.1. Chromosome Number

Chromosome number is a characteristic of species. It is constant in each cell of all normal individuals of a single species. It is as important a datum for a species as any other characteristic considered significantly stable to merit taxonomic significance (Garber, 1979).

Though chromosome number is generally constant for a species, variable number of chromosome was found in some mammals, which was attributed mainly to Robertsonian translocation (Wahrman *et al*., 1969; Searle *et al*., 1990; Nevo *et al*., 1994). Robertsonian translocation is the result either of the fusion of two centromeres into one or the fission of one centromere into two. In mammals, centric fusions are generally regarded to be more common than fission, but different opinions exist among scientists (Fredga, 1977). Centric fusion decreases chromosome number since two telocentric chromosomes are combined into one metacentric chromosome whereas centric fission increases chromosome number as a metacentric chromosome dissociates into two telocentrics.

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, called tandem fusion, is another source of variation in chromosome number within a taxon in mammals (Gibson, 1984). 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).

Though allopolyploidy is almost entirely restricted to plants (White, 1968a), it also causes chromosome number variation in animals. For example, *Tympanoctomy barrenae* from Argentina is found to be tetraploid, which is the first tetraploid rodent (Milton, 2000).

Still another source of variation in chromosome number is seen occasionally in mammals, and more frequently in birds and reptiles (Gibson, 1984). This is the presence of extra, often very small chromosomes, called B chromosomes or supernumerary chromosomes. The origin of these chromosomes is unknown, but they may be remnants of chromosomal rearrangements (White, 1973).

Generally, mammals have a wide range of variation in chromosome number, and the widest range being in Rodentia (Fredga, 1977). In mammals, the lowest chromosome number, 2n=6, was found in a small deer, Indian muntjac (Wurster and Benirschke, 1970) and the highest (2n=102) in *Tympanoctomy barrenae* from Argentina (Milton, 2000).

However, so far, the diploid chromosome number of *T. splendens* has been reported to be 2n=48, though other chromosome variations (variations in morphology of the Y-chromosome, fundamental number, and number of C-positive autosomes) have been observed (Matthey, 1956; 1967 cited in Baskevich *et al*., 1993; Aniskin *et al*., 1997; Lavrenchenko *et al*., 1997).

### 2.1.2. Autosomal Fundamental Number

According to Robbins and Baker (1978), the nombre fundamental (NF) or the fundamental number (number of chromosome arms) has two definitions. In the first definition, it includes all the arms of the sex and the autosomal chromosomes, whereas in the second definition, it includes only the arms of the autosomal chromosomes and is referred to as autosomal fundamental number (NFa).

Variation in the number of chromosome arms is common in some rodent species. For example, the autosomal fundamental number of *Myomys albipes* from Ethiopia ranges from 50-53 (Corti *et al*., 1999), in *Peromyscus maniculatus* from Arizona ranges from 62-80 (Duffey, 1972), in *Ctenomys flamarioni* ranges from 50-78 (de Freitas, 1994).

The autosomal fundamental number of *T. splendens* also showed variation, NFa=51-86 (Matthey, 1956; 1967 cited in Baskevich *et al*., 1993; Baskevich *et al*., 1993; Aniskin *et al*., 1997; Lavrenchenko *et al*.,1997). Though Baskevich *et al*. (1993) had suggested that the number of two-armed chromosomes decreases with the increase in aridity from the north to the south, Aniskin *et al*. (1997) reported that their data did not confirm the earlier suggestion.

The classic explanation for such a varying number of chromosome arms but the same number of chromosomes was to be pericentric inversions or unequal reciprocal translocations (Fredga, 1977; Baskevich *et al*., 1993). However, such karyotypic variation in some animals was suggested to be due to variations in constitutive heterochromatin (Duffey, 1972; Baskevich *et al*., 1993), which was resulted either by loss or amplification processes (Deaven *et al*., 1977).

### 2.1.3. Chromosome Morphology

Chromosome morphology is usually studied at the metaphase of mitosis, when chromosomes become contracted to the maximum or nearly so (Stebbins, 1971). Chromosomes are arrested at metaphase by treating with spindle disrupting or inhibiting chemicals such as colchicine, colcemid, vinblastine, bromonapthalene, 8-hydroxyquinoline or griseofulvin (Jackson, 1971).

Mitotic metaphase chromosomes have characteristic morphological features, which allow cytogeneticists to distinguish one chromosome set from another, as well as one chromosome from another. Studying the morphology of chromosomes and classifying them accordingly helps to reach understanding of how new karyotype evolution has proceeded and to find correlations with the evolution of other systematic characteristics such as anatomical, biochemical and behavioral (Levan *et al*., 1964).

These topological features of chromosomes include chromosome length, centromeric position, secondary constriction and satellites, and nucleolar organizer region. In addition to chromosome

number, these topological features of chromosomes are important in taxonomic studies (Stace, 1980).

### 2.1.3.1. Chromosome Length

The length of metaphase chromosome is a characteristic property and chromosomes may arbitrarily be classified as long (>10 μm), medium (4-8 μm) or short (< 2 μm) (Bernard, 1976 cited in Mohamed Abate, 2004). However, measuring the length of a chromosome from different cells of the same individual or different individuals of the same species may not give the same figure, because the degree of contraction of the chromosome varies depending partly on different conditions of chromosome treatments. The concentration and time of exposure of spindle inhibitors like colchicine (Ronne and Wulf, 1980), and the concentration and type of salt used during hypotonic treatment are the major determinants of the average chromosome length in standard preparations (Ronne, 1989). Though measurements of chromosome length are sometimes inexact, they provide at least the best information on the relative size of the chromosome (Cohn, 1969). In mammals chromosome size is inversely proportional to chromosome number since the DNA content is relatively constant (Fredga, 1977). The size measurement of the chromosomes of *T. splendens* has not been reported yet.

### 2.1.3.2. The Centromere

The centromere is the region of a chromosome to which spindle fibers attach. The centromere region usually appears to be constricted, and the position of this constriction is the most useful landmark, which is characterized by great constancy (Levan *et al*., 1964). Although Levan *et al.* (1964) recommended the definition of the relative position of the centromere on a chromosome based on the arm ratio (r=l/s; l=long arm and s=short arm) as median point (M), median region (m), submedian region (sm), subterminal region (st), terminal region (t) and terminal point (T) with arm ratios corresponding, respectively as 1.0, 1.0-1.7, 1.7-3.0, 3.0-7.0, 7.0-∞, and ∞; still the following terminologies are in use to describe a chromosome. Telocentric: centromere located at one end of the chromosome. Acrocentric: centromere located near one end of the chromosome so that it contains one long and one very short arm (practically, acrocentics are equivalent to telocentrics)**.** Submetacentric: centromere located near one end of the chromosome than the other

so that the two arms are distinctly unequal, but less than in acrocentric situation. Metacentric: centromere located at or near to the middle of the chromosome so that its arms are nearly or quite equal in length.

#### 2.1.3.3. Secondary Constriction and Satellites

Some chromosomes, in addition to the primary constriction formed by the centromere, show secondary constrictions. Unlike the centromere, secondary constriction is not the characteristic of the whole chromosomes in a cell, it could only be found in a few chromosomes, even it may be absent in the chromosome region where the constriction is normally seen depending upon the dynamics of chromosome condensation (Busch and Smetana, 1970 cited in Berns and Cheng, 1971). It is generally assumed that nucleolus associated chromosomal secondary constrictions are nucleolar organizers and these regions contain ribosomal RNA genes (Miller *et al*., 1976; Graphodatsky, 1989; Halnan, 1989).

Satellites are formed by secondary constrictions on the nucleolar organizer chromosomes. Thus, the size of satellites can vary considerably depending on the relative position of the secondary constrictions on a chromosome. They are also found to be very variable in appearance as sometimes being very conspicuous and at other times indiscernible and thus it is not always possible to obtain consistent results (Stace, 1980).

#### 2.1.3.4. The Nucleolar Organizer Region

Nucleolar organizer region **(**NOR) is the ribosomal DNA element of a genome; that is, it is region of a chromosome, which contains ribosomal RNA genes (Jordan and Luck, 1976; Schwarzacher and Wachtler, 1983; Halnan, 1989). Nucleolus is a structure containing the nucleolar organizer region and the accumulated material around it, most notably the rRNAs and their precursors (Warner, 1979).

Different organisms have different number of nucleolar organizer chromosomes, and the position of NORs is highly specific in a chromosome set. For example, the NORs of dog, fox, and raccoon dog are found to be located on the telomers of three chromosome pairs, while in most species of Mustelidae the NOR is found in the secondary constriction of a single autosomal pair

(Graphodatsky, 1989). On the other hand, the NORs of *T. splendens* are found to be localized at the telomeric ends of the short heterochromatic arms of the first and second autosomal pairs (Baskevich *et al.* 1993). Thus, the number and position of NORs in a chromosome set can be used as landmarks for cytogenetic analysis.

### 2.1.4. Chromosome Banding

Special chromosome staining procedures have revealed specific sets of intricate bands in many different organisms. A chromosome band is a manifestation of a segment of chromatin that has relatively homogeneous and distinctive functional and structural features over a large enough stretch for it to be visually distinguished from its neighboring segments (Bickmore and Craig, 1997). The position and size of the bands is highly chromosome specific. Bands that show a strong staining reaction are referred to as positive bands and that show no staining reaction are referred to as negative bands.

Many suggestions have been made to explain the mechanism of chromosome banding and most of them center around DNA-protein-dye interaction and variation of base pair composition of the chromosome. Comings *et al*. (1973), and Comings and Avelion (1974) suggested that certain DNA-nonhistone protein interactions play an important role in chromosome banding (C- and G-banding). Burkholder and Duczek (1982) examined the effect of standard G-, C-, R-banding techniques on histone and non-histone proteins from isolated, fixed and air-dried chromosomes and each of the banding treatments induced characteristic alterations in chromosome proteins. Treatments inducing the same type of banding had similar effects on chromosome proteins while treatments inducing different types of banding showed different effects. Thus, they suggested that these protein alterations may have an important influence on the induction of chromosome bands. van Duijn *et al*. (1985) explained Giemsa banding mechanism as interaction between a hydrophobic dye complex, the supercoiled helical DNA, and the denatured histone core of the nucleosomes. Schreck *et al*., (1973) and Halnan (1989) suggested that chromosome banding techniques are sensitive to variations in base composition of DNA However, according to Saitoh and Laemmli (1994), the alternating pattern of AT-and GC-rich bands along a chromosome is unlikely to be due to a simple linear variation of the base composition of DNA and they suggested that bands arise from a differential folding path of a highly AT-rich (>65%) scaffold.

Though the mechanisms of differential staining are not clear, the use of banding makes it possible to identify homologous chromosome, to detect the homologous parts of chromosomes in the compared forms and the identification of chromosome rearrangements, and it revolutionized comparative cytogenetics of mammals (Graphodatsky, 1989). Chromosome banding is also important in understanding patterns of evolution of chromosomes and relationship among taxa that exhibit different chromosomal conditions (Qumsiyeh *et al*., 1990).

Several methods of chromosome banding techniques have been developed. The most commonly used methods of chromosome banding in mammals are C-(centromere) and G-(Giemsa) banding. Standard Giemsa staining is also routinely used for making a karyotype.

#### 2.1.4.1. C-banding

C-banding reveals constitutive heterochromatin, which in mammals is located around the centromere (Pardue and Gall, 1970). In different species it can also be located at different sites including telomeric, satellite, and interstitial regions of chromosomes. C-banding technique is very useful for identifying homologous chromosomes, because they have heterochromatin at the same site (Hilwig and Groop, 1972), and for identifying the Y-chromosome of animals.

Heterochromatin is traditionally classified into two: constitutive and facultative. Constitutive heterochromatin is thought to be present throughout the life of an organism whereas facultative heterochromatin is defined as being under developmental control and capable of reverting to euchromatin form (Bickmore and Craig, 1997). Constitutive heterochromatin can be detected by C-banding. On the other hand, the facultative heterochromatin, for example, X-chromosome (inactive X-chromosome) in female somatic cells of mammals does not have C-band (Kanda, 1973). According to Hsu (1975), generally, constitutive heterochromatin: exists in all mammals, replicates late during the S-phase, contains highly repeated DNA sequence, is usually centromeric in location, is highly polymorphic in size, and probably never transcribed.

Constitutive heterochromatin is generally considered to be genetically inert, but it can have an effect on gene action, chromosome behavior, or the phenotype (Stebbins, 1966; Garber, 1979). There are many hypotheses on the role of heterochromatin in the life of organisms. For example, Hsu (1975) proposed the bodyguard hypothesis, which states that constitutive heterochromatin is

used by cells as a bodyguard to protect the vital euchromatin by forming a layer of dispensable shield on the outer surface of the nucleus. Yunis *et al*. (1971) also suggested that heterochromatin may protect the vital areas of the genome like the centromere and NOR from external forces. However, none of them completely explains the diversity of heterochromatin, which occurs in modern species (Graphodatsky, 1989).

Graphodatsky (1989) emphasized that all mammals are virtually characterized by polymorphism in both location and size of heterochromatic blocks revealed by C-banding. Indeed, Aniskin *et al*, (1997) found heterochromatin polymorphism in *T. splendens*, and they suggested that the variations might be associated with high lability of the species allowing rapid adaptation to various habitats.

#### 2.1.4.2. G-banding

According to Halnan (1989), G-banging technique reveals variation in GC-rich verses AT-rich regions throughout the length of the arms of chromosomes. Giemsa dark (G) bands are late replicating and AT-rich, and can also be revealed with fluorochromes such as quinacrine (Q-bands) (Saitoh and Laemmli, 1994).

G-band homology has been shown to reflect genetic homology (Lalley *et al*., 1978; Sawyer and Hozier, 1986), be compared between individuals, populations, species, genera, and even higher taxonomic categories in mammals (Baker *et al*., 1987 cited in Qumsiyeh *et al*., 1990). However, Graphodatsky (1989) warns that comparing chromosome homology between chromosome regions, which may lack genetic similarity, is dangerous and banding data alone is not enough in comparing karyotypes with extensive rearrangements. The smaller the differences between the karyotypes compared, the more reliable the identification of the G-homologous chromosome regions revealed by banding (Graphodatsky, 1989).

Many comparative investigations using G-banding have been conducted on Rodentia mostly on the karyotypes of closely related species and a large number of homologous elements have been shown in their karyotypes (Graphodatsky, 1989; Qumsiyeh *et al*., 1990; Aniskin *et al*., 1997; Corti *et al*., 1999). Thus, the comparative study of G-banding can be used to derive a cladistically based chromosome phylogeny (Ingram *et al*., 2004).

Analysis of chromosome G-banding patterns of *T. splendens* and that of its closely related species, *T. macrocephalus*, showed that their karyotype formation involved euchromatic rearrangements and originated from a common ancestoral karyotype (Aniskin *et al*., 1997).

#### 2.1.4.3. Silver Staining

The chromosome regions that carry the rRNA genes, the nucleolar organizer regions, can be stained preferentially by silver staining method (Goodpasture and Bloom, 1975). Miller *et al*. (1976) also suggested that silver staining detects chromosome regions that functioned as nucleolus organizers in the preceded interphase and produced rRNA.

### 2.1.5. Karyotype

A karyotype is a systematized arrangement of the chromosomes of a single cell prepared either by drawing or by cutting them from a photograph and sticking on a paper (Halnan, 1989). If the preparation was made from a normal individual, the karyotype is considered to be typical of the species. It is also the phenotypic appearance of the somatic chromosomes in contrast to their genotype (Levitsky, 1924; 1931 cited in Sharma, 1991). Some authors include a pen-ink ruler drawing of the chromosomes, called an idiogram, arranged in duplication of the karyotype, and it indicates landmarks in the bands. In general, karyotype is a species characteristic, but in some orders, like seals and whales, many distantly related species have apparently identical karyotypes (Fredga, 1977) while in other orders, like rodents, different types of karyotypes exist within a species.

A huge number of karyotypic variations found in extant species are unmistakable evidence that there is association between evolutionary processes and karyotypic changes (Fredga, 1977). Most groups of animals, even the most closely related species, differ in their karyotypes, and this suggests that in many instances the origin of karyotypic differences and the origin of new species may have been related events (White, 1968b). Bush *et al*. (1977) also found strong correlation between rate of chromosomal evolution and average rates of speciation in mammals. Karyotypes have proven to be valuable data for evolutionary and systematic studies (Robbins and Baker, 1978).

Investigation of chromosome rearrangements at the level of intraspecies polymorphism suggests that many rearrangements lack pronounced phenotypic manifestation, being probably neutral for selection (Graphodatsky, 1989). Karyotypic similarity among related organisms indicates phylogenetic relationship, since the similarity does not originate *de novo* among unrelated species (Arnason, 1972).

According to Aniskin *et al*. (1997), the characterization of any natural population of rodents must include karyotype analysis. Baskevich *et al*. (1993) also found the karyotype of burrowing mammals to be interesting from the cytogenetic point of view.

# 3. OBJECTIVES OF THE STUDY

## 3.1. General objective

The general objective of this study is to study chromosomes of *T. splendens* from different localities in Ethiopia.

## 3.2. Specific Objectives

The specific objectives of this study are to:

- determine the somatic chromosome number of *T. splendens* from different localities
- describe chromosome morphology including autosomal fundamental number of *T. splendens* from different localities
- determine constitutive heterochromatin distribution of *T. splendens* through C-banding technique
- construct and compare karyotypes of different populations of *T. splendens.*

# 4. MATERIALS AND METHODS

## 4.1. Collection of Specimens

### 4.1.1. Study Localities

Specimens were collected from nine localities in Ethiopia (Fig. 2 and Table 1). The geographic location and altitude of each locality and the number of specimens collected are presented in Table 1. The localities were selected with reference to their geographic location, presence and adequate representation of the species (Sewnet Mengistu and Afework Bekele, 2004).

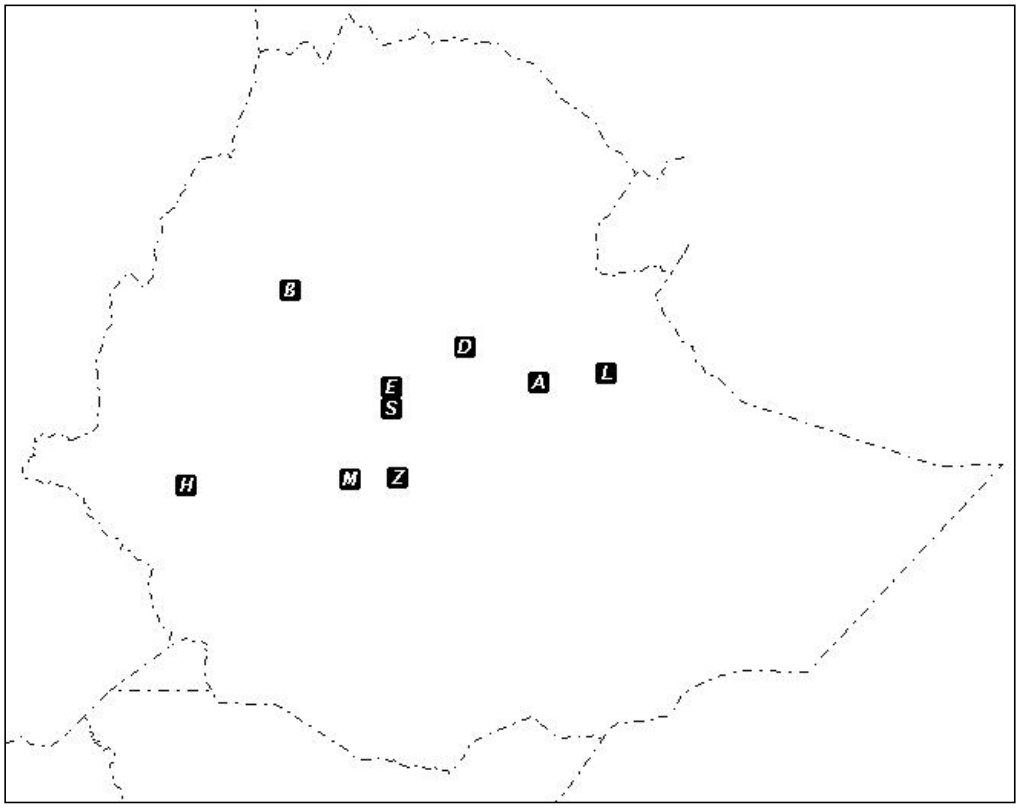



Fig. 2. Map of Ethiopia showing *T. splendens* specimen collection sites. B, Bure; E, Entoto; S, Sebeta; D, Debre Sina; M, Mugo; Z, Ziway; A, Asebe Teferi; L, Alemaya; and H, Masha.

Table 1. The geographic location and altitude of each locality and the number of *T. splendens* collected.

| Locality | Geographic location | Altitude (m) | Male | Female | Total |
|---|---|---|---|---|---|
| Entoto | 9⁰ 02'30"N, 38⁰35'50"E | 2982 | 1 | 2 | 3 |
| Sebeta | 8⁰55'N, 38⁰38'E | 2200 | 1 | 5 | 6 |
| Ziway | 7⁰52'25"N, 38⁰43'44"E | 1665 | 1 | 1 | 2 |
| Debre Sina | 9⁰50'00"N, 39⁰16'25"E | 1900 | 1 | 2 | 3 |
| Asebe Teferi | 9⁰ 04'30"N, 40⁰51'51"E | 1820 | 2 | 1 | 3 |
| Mugo | 7⁰50'18"N, 37⁰59'06"E | 2974 | 1 | 2 | 3 |
| Bure | 10⁰40'30'N, 37⁰04'20"E | 2670 | 1 | 2 | 3 |
| Alemaya | 9⁰27'N, 42⁰01'E | 2125 | 2 | 1 | 3 |
| Masha | 7⁰43'N, 35⁰28'E | 2325 | 2 | - | 2 |
| Total | | | 12 | 16 | 28 |

### 4.1.2. Specimens Trapping

Specimens were collected from January to October 2004. The presence of mole rats in each locality was confirmed by surveying the presence of aboveground fresh mole-hill. Victor gopher traps were used (Sewnet Mengistu and Afework Bekele, 2003). Traps were inserted into the burrow and tied to a wooden stick partly entrenched in the ground to prevent them from being taken inside the burrow. Live-trapped mole rats from Entoto and Sebeta were transported to the Genetics Laboratory of Biology Department, Addis Ababa University, for chromosome preparation. In the case of the other localities, chromosome preparations were made in the field.

### 4.2. Somatic Chromosome Study

Chromosome preparations for somatic chromosome analysis were obtained from bone marrow cells by Baker's (1970) method, cited in Robbins and Baker (1978), with some modification.

#### 4.2.1. Metaphase Chromosome Slide Preparation

Individual mole rats were weighed using a hand-balance, injected with 0.05% colchicine at the rate of 1ml per 100 gm body weight, and left in a rat cage for one and half hour. The animal was then etherized and killed; the upper hind leg bones (femurs) were dissected and crushed in a Petri dish containing about 5 ml of hypotonic solution of 0.075 M KCl. Using Pasteur pipette, the cell suspension was transferred from the Petri dish to a centrifuge tube, and incubated at room temperature for 20 minutes. The cell suspension was centrifuged at 1000 rpm for 10 minutes, the supernatant was discarded, and the pellet was suspended in about 5ml freshly prepared fixative (3 parts methanol and 1 part acetic acid). After at least 10 minutes of fixation at room temperature the suspension was centrifuged at 1000 rpm for 10 minutes. After 3 rounds of suspension in fixative followed by centrifugation, the pellet was re-suspended in a small volume (<0.5ml) of fixative. A test-slide was prepared by splashing 2 or 3 drops of fixed cell suspension from a height of 0.5m or more onto the slide. After staining with aceto-orcein, the slide was covered with a cover slip and observed under light microscope. When good metaphase chromosome spreads were observed, more slides were prepared from the remaining cell suspension. The slides were then air-dried and stored.

#### 4.2.2. Conventional Giemsa Staining

Unstained air-dried slides were screened under a phase contrast microscope to select those with well spread metaphase chromosomes. The selected slides were stained in 4% Giemsa in Sorensen's phosphate buffer (pH=6.8) for 10 minutes or more until the chromosomes get stained satisfactorily, the slides were rinsed in two changes of distilled water, air-dried and mounted in DPX (Gustashaw, 1991).

### 4.2.3. C-banding

Sumner's (1972) method was used to detect the amount and distribution of constitutive heterochromatin C-bands in the chromosomes. Air-dried chromosome preparation slides were treated with 0.2N HCl for 2 hours at room temperature, and rinsed in 3 changes of distilled water. They were then treated in 5% solution of $Ba(OH)_2$ for 10 minutes at $60^0$C. After being rinsed in several changes of distilled water, the slides were incubated in 2xSSC at $60^0$C for one hour and rinsed in distilled water. Finally, the slides were stained with 4% Giemsa in Sorensen's phosphate buffer (pH=6.8) for one and half an hour or more until chromosomes get stained well, rinsed in distilled water, air-dried and mounted in DPX (mixture of Distyrene, plactcister, and Xylene)

### 4.2.4. Karyotype Analysis

Photomicrographs of good metaphase chromosome spreads were taken from both Giemsa stained and C-banded slides using a camera fitted into a microscope with a magnification of x100 objective. The number of animals studied from each locality and the number of slides and metaphase cells analyzed from each animal are shown in Appendix 2. Chromosomes were described and characterized using photomicrographs and direct observation under the microscope. The karyotypes were constructed by arranging putative homologous chromosomes into pairs using centromeric position and chromosome size as criteria. The total length and the arm lengths of the sex chromosomes and total length of the largest autosome were measured from photomicrographs using a ruler. Then, the arm ratios, the average chromosome lengths and standard deviations were calculated (Table 2).

# 5. RESULTS

## 5.1. Chromosome Number

There was no difference in chromosome number among the specimens of *T. splendens* considered in this study. All the specimens from the nine localities had a diploid chromosome number of 2n=48 (Figs. 3(A-D) & 4(A-E) and Table 3).

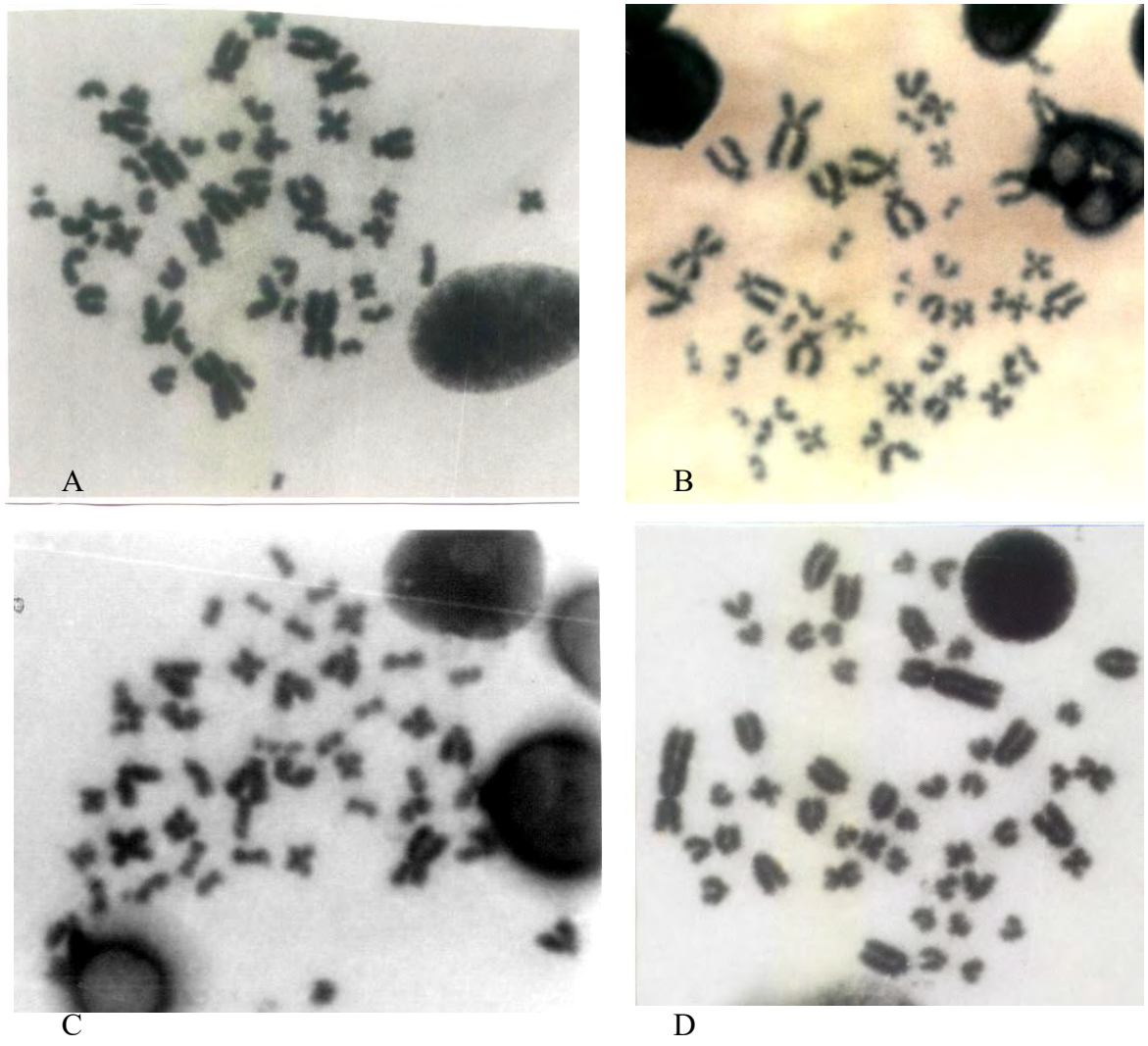



Fig. 3. Mitotic metaphase chromosome (2n=48) of *T. splendens* from different localities in Ethiopia: (A) Mugo female, (B) Masha male, (C) Bure male, and (D) Entoto female.

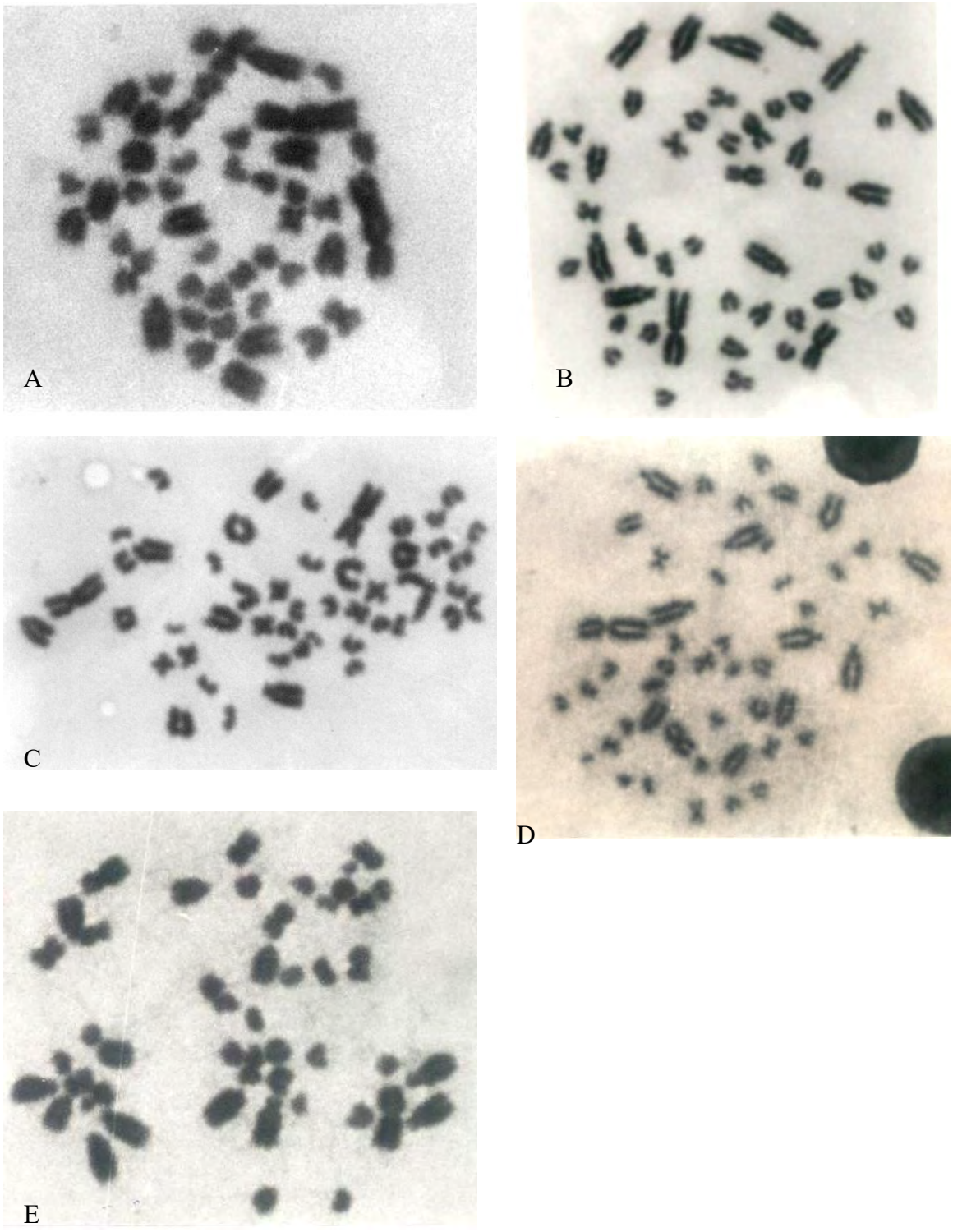



Fig. 4. Mitotic metaphase chromosome (2n=48) of *T. splendens* from different localities in Ethiopia: (A) Sebeta female, and (B) Ziway male, (C) Asebe Teferi female, (D) Alemaya male, and (E) Debre Sina male.

### 5.2. Karyotype Description

Though it was difficult to take measurements of chromosome length from small chromosomes, an attempt was made to take measurements from photomicrographs of some large chromosomes. Based on photomicrographs and visual analysis under the microscope, description of chromosome morphology and karyotype analysis were made (Figs. 5-9 and Tables 2 & 3). Six different karyotypic forms of *T. splendens* were found from the nine localities studied.

Standard Giemsa stained chromosomes of, *T. splendens* from Mugo, Masha and Bure showed virtually similar karyotypes, especially with regard to the autosomal complements (Figs. 5(A & B) & 6(A), and Tables 2 & 3). The autosomal complements of *T. splendens* from these localities consisted of 5 pairs of medium-sized metacentric, 5 pairs of large subtelocentric and 13 pairs of medium- to small-sized acrocentric chromosomes. The X-chromosome was the largest and metacentric in all these localities. The Y-chromosome was also a large metacentric, but smaller than the X-chromosome. Y-chromosomes from Bure and Masha were perfect metacentric (centromere at median point). The autosomal fundamental number (NFa) of *T. splendens* from all these localities was 66. Thus, the karyotypic formula for the three localities was 12m + 10st + 26a.

Similarly, *T. splendens* from Entoto and Sebeta showed almost similar karyotypes (Figs. 6(B) & 7(A), and Tables 2 & 3). The karyotypes of *T. splendens* from these localities comprised of 3 pairs of metacentric (a pair of which were larger and distinguishable from the other two pairs), a pair of large subtelocentric, 19 pairs of medium- to small-sized acrocentric autosomes, and a pair of sex chromosomes. The X-chromosome was the largest as that of the other localities but uniquely submetacentric for these two localities. The Y-chromosomes was large (uniquely, larger than the largest autosome) and metacentric in Sebeta but, as no male specimen was studied from Entoto, it was not possible to describe the Y-chromosome of Entoto. The autosomal fundamental number was 54, and the karyotypic formula was 7m + 1 sm + 2st + 38a for specimens from both localities.

The karyotypes of *T. splendens* from three localities, namely: Ziway, Asebe Teferi and Alemaya showed general similarity but each has certain distinguishing features (Figs. 7 (B) & 8, and Tables 2 & 3). *T. splendens* from the three localities had 3 pairs of medium-sized metacentric

autosomes (in Ziway, a pair of which was larger and distinguishable from the other two pairs) and a pair of metacentric sex chromosomes. However, with respect to the rest autosomal components, *T. splendens* from these localities had different numbers of large subtelocentric and medium- to small-sized acrocentric chromosomes. *T. splendens* from Ziway had 6 pairs of subtelocentrics and 14 pairs of acrocentrics whereas that of Asebe Teferi had 2 pairs of subtelocentrics and 18 pairs of acrocentrics. The animal from Alemaya had 3 subtelocentric and 17 acrocentric pairs. Thus, the autosomal fundamental number varied accordingly as 64, 56, and 58, for Ziway, Asebe Teferi, and Alemaya, respectively. Thus, the karyotypic formulae of *T. splendens* from Ziway, Asebe Teferi, and Alemaya were (8m + 12st + 28a), (8m + 4st + 36a), and (8m + 6st + 34a), respectively.

*Tachyoryctes splendens* from Debre Sina had a unique karyotype, which was composed of 4 pair medium-sized metacentric, 4 pair subtelocentric, 15 pairs acrocentric autosomes, and a pair of metacentric sex chromosomes (Fig. 9). The X- chromosome was the largest in the complement and the Y-chromosome was slightly smaller than the largest autosome. The autosomal fundamental number was 62, and the karyotypic formula was 10m + 8st + 30a.

An attempt was made to take absolute chromosome length measurements from the largest autosome and from the sex chromosomes of *T. splendens* in each locality. *T. splendens* from Ziway had the largest autosome with an average length of 5.7± 0.91 µm and that of Bure and Debre Sina had the smallest of all the largest autosomes with a length of 4.1 µm. The largest autosomes were consistently subtelocentric with a very short second arm. Compared to all other localities, the X-chromosome from Entoto and Sebeta was the largest, with an average length of 10 µm and that from Debre Sina was the smallest, with a length of 5.0 µm. Similarly, the largest Y-chromosome was from Sebeta while the smallest was from Mugo with a length of 8.6 µm and 3.4± 0.32 µm, respectively.

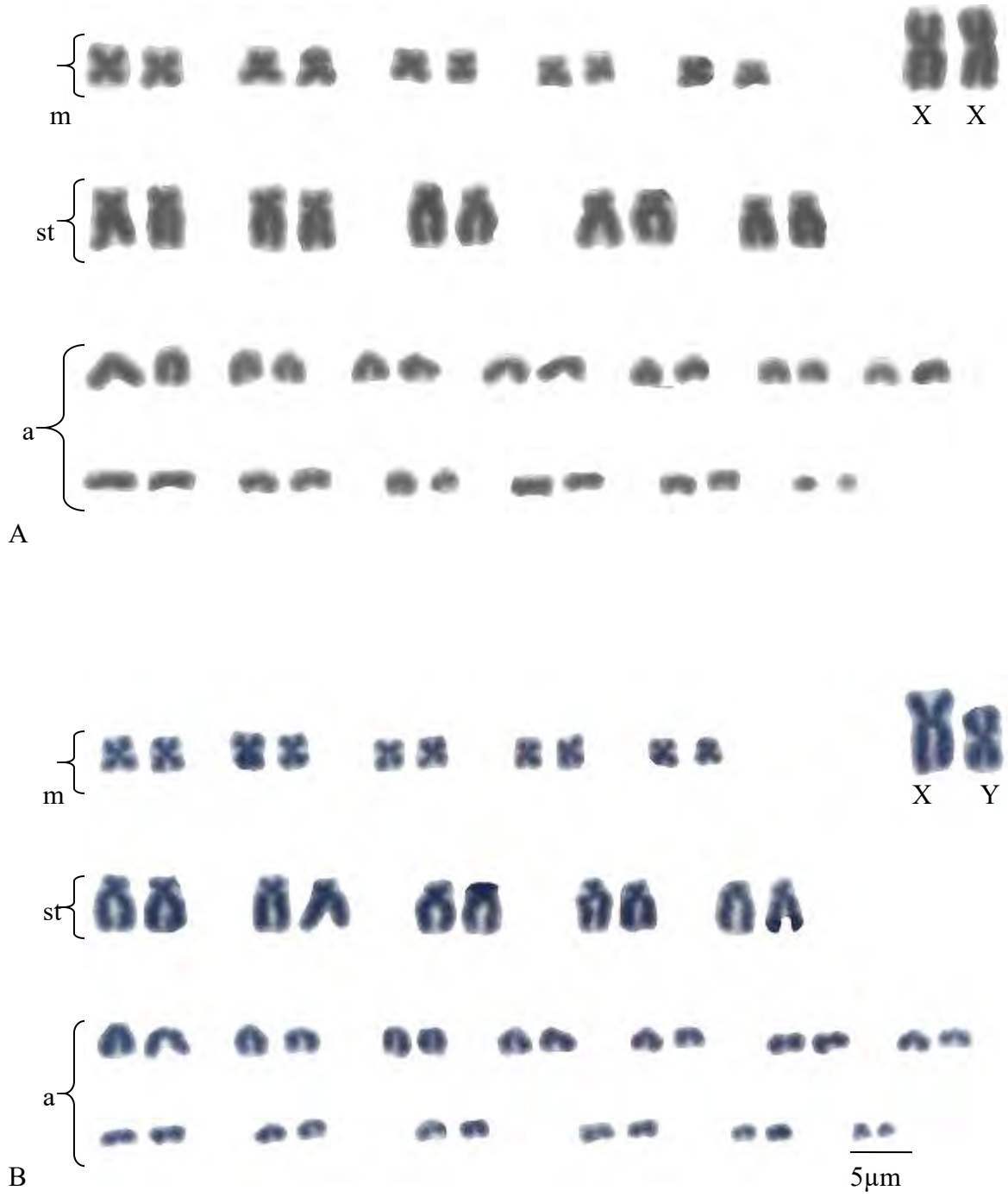



Fig. 5. Karyotypes of *Tachyoryctes splendens* using standard Giemsa staining: (A) Mugo female and (B) Masha male. Bar = 5µm.

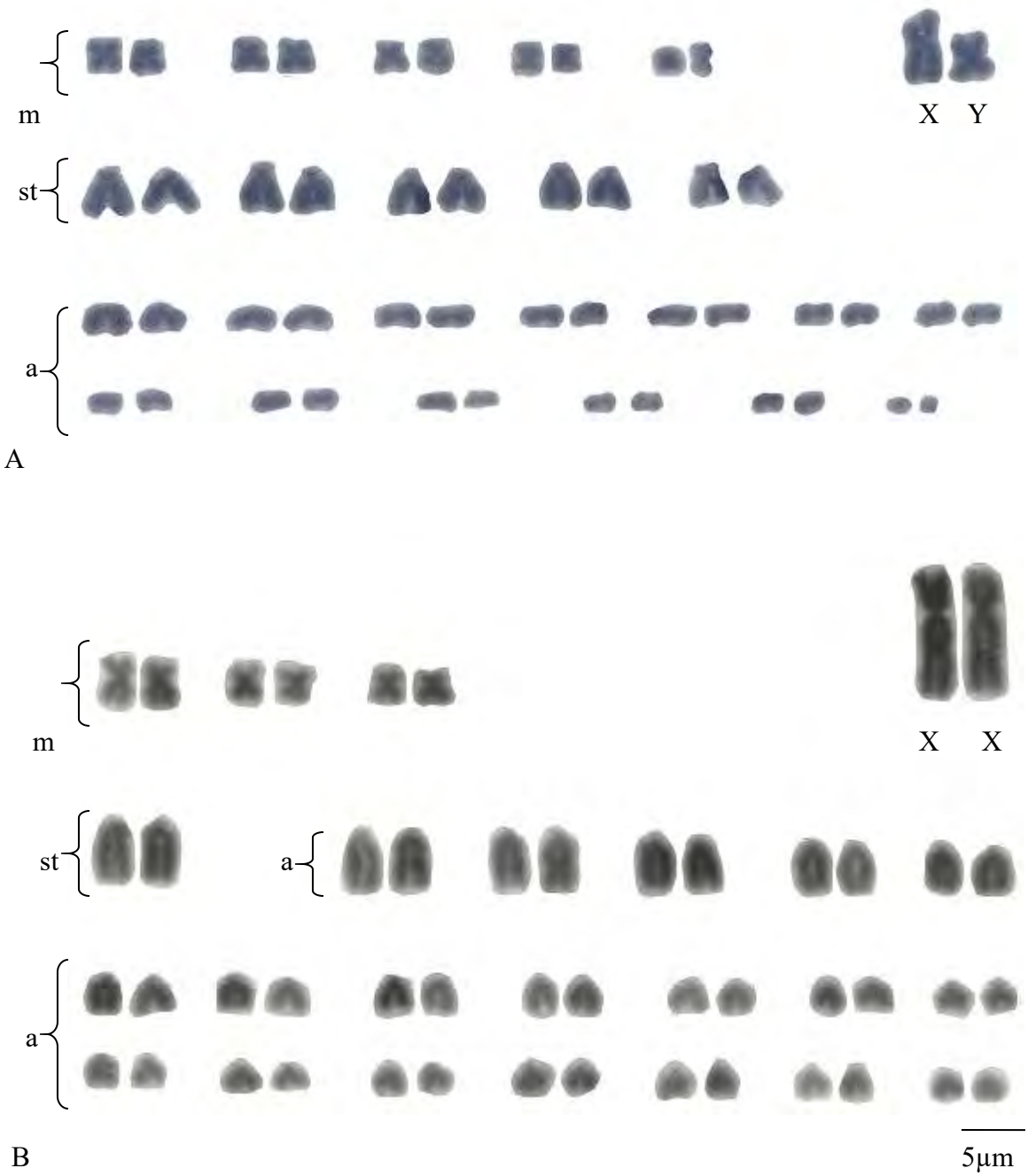



Fig. 6. Karyotypes of *Tachyoryctes splendens* using standard Giemsa staining: (A) Bure male and (B) Entoto female. Bar = 5µm.

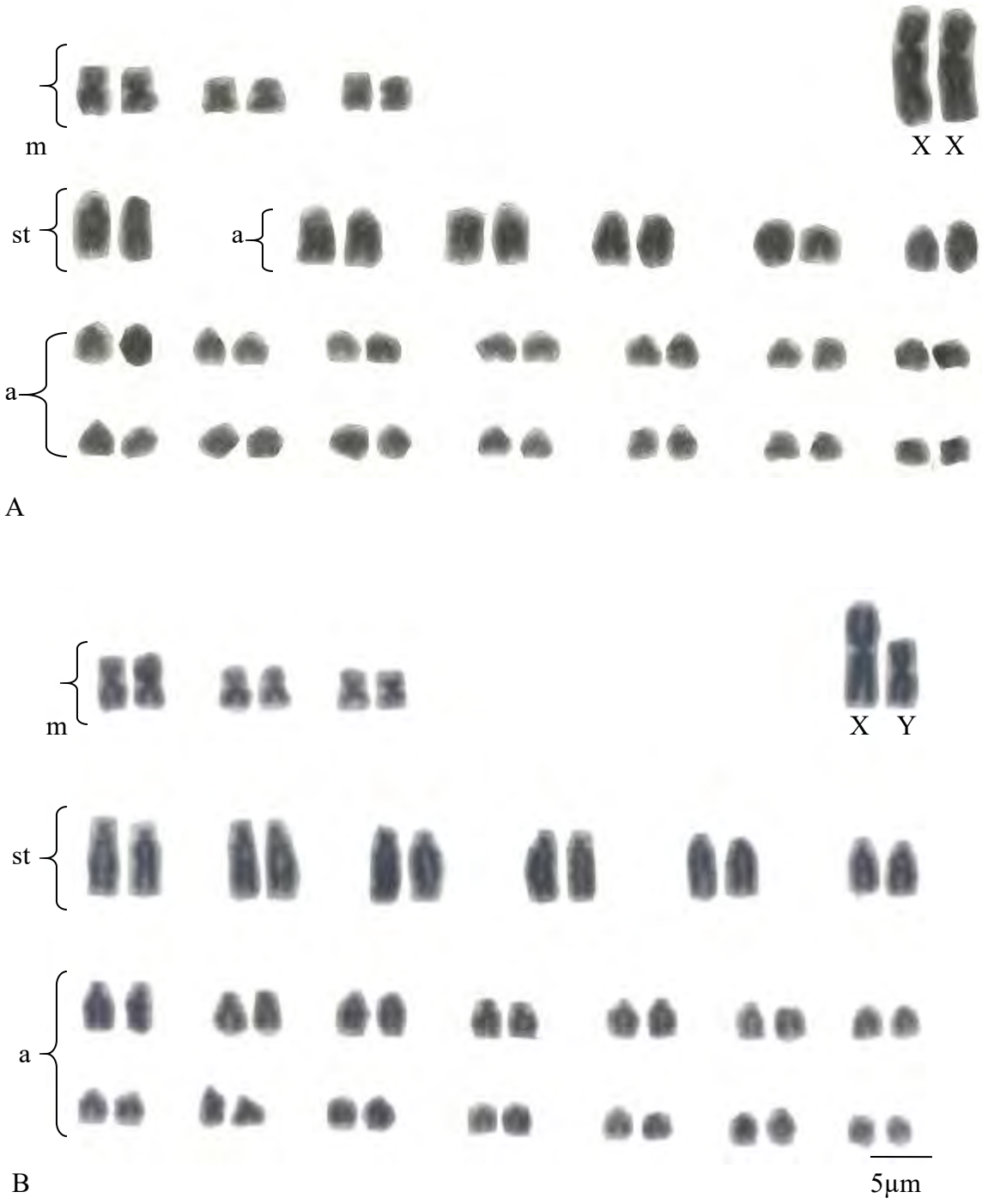



Fig. 7. Karyotypes of *Tachyoryctes splendens* using standard Giemsa staining: (A) Sebeta female and (B) Ziway male. Bar = 5µm.

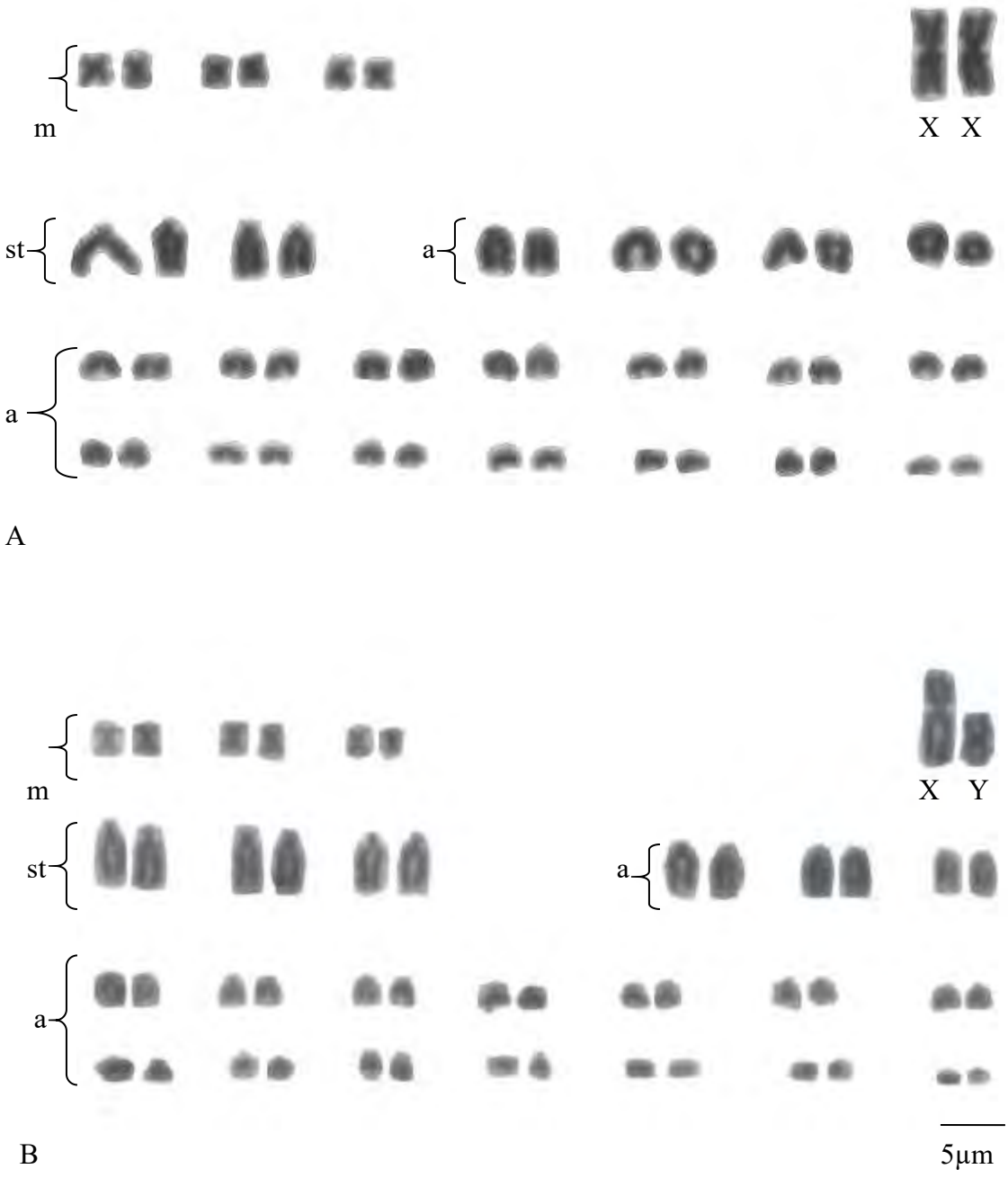



Fig. 8. Karyotypes of *Tachyoryctes splendens* using standard Giemsa staining: (A) Asebe Teferi female and (B) Alemaya male. Bar = 5µm.

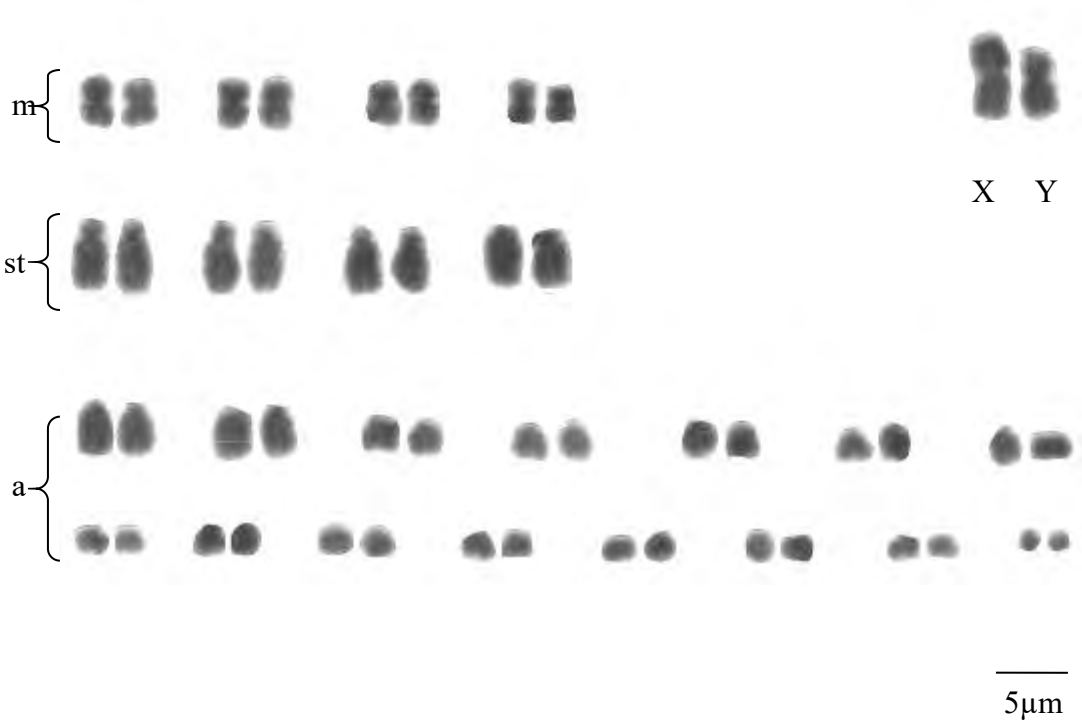



Fig. 9. Karyotype of male *Tachyoryctes splendens* from Debre Sina using standard Giemsa staining. Bar = 5µm.

Table 2**.** Mean lengths of the largest autosome and the sex chromosomes, means of the arm ratios (r=L/S) and centromeric position of the sex chromosomes of *T. splendens* from different localities.

| Locality | Sex | X-chromosome | | | Y-chromosome | | | Largest autosome |
|---|---|---|---|---|---|---|---|---|
| | | Length (µm) | r=L/S | Centromere position | Length (µm) | r=L/S | Centromere position | Length (µm) |
| Mugo | M | 5.9±0.00 | 1.6 | m | 3.4±0.32 | 1.3 | m | 4.8±0.32 |
| | F | 7.0±0.84 | 1.3 | m | – | – | – | 5.3±0.75 |
| Masha | M | 7.3±0.84 | 1.6 | m | 4.4±0.80 | 1.0 | M | 4.9±0.68 |
| Bure | M | 6.4 | 1.3 | m | 3.6 | 1.0 | M | 4.1 |
| Ziway | M | 7.4±1.10 | 1.2 | m | 5.1±0.55 | 1.3 | m | 5.7±0.91 |
| | F | 7.3±0.64 | 1.5 | m | – | – | – | 5.2±0.26 |
| Alemaya | M | 6.7±0.59 | 1.3 | m | 4.4±0.52 | 1.5 | m | 4.6±0.20 |
| | F | 6.8±0.00 | 1.5 | m | – | – | – | 4.5±0.00 |
| AsebeTeferi | M | 6.4±1.62 | 1.4 | m | 4.1±0.83 | 1.3 | m | 4.5±0.83 |
| | F | 7.3±1.54 | 1.3 | m | – | – | – | 4.5±0.79 |
| Entoto | F | 10.0±1.25 | 2.1 | sm | – | – | – | 5.0±0.50 |
| Sebeta | M | 10.0 | 2.1 | sm | 8.6 | 1.4 | m | 5.0 |
| | F | 9.7±1.53 | 2.2 | sm | – | – | – | 5.4±0.99 |
| Debre Sina | M | 5.0 | 1.2 | m | 3.6 | 1.7 | m | 4.1 |

Table 3. Karyological data of *T. splendens* from different localities in Ethiopia showing the diploid chromosome number (2n), autosomal fundamental number (NFa), centromeric position of the sex chromosomes and the number of autosomes with different centromeric positions (m, st & T and/or t).

| Locality | 2n | NFa | X– | Y– | m | st | T and/or t |
|---|---|---|---|---|---|---|---|
| Mugo | 48 | 66 | m | m | 10 | 10 | 26 |
| Masha | 48 | 66 | m | M | 10 | 10 | 26 |
| Bure | 48 | 66 | m | M | 10 | 10 | 26 |
| Ziway | 48 | 64 | m | m | 6 | 12 | 28 |
| Alemaya | 48 | 58 | m | m | 6 | 6 | 34 |
| Asebe Teferi | 48 | 56 | m | m | 6 | 4 | 36 |
| Entoto | 48 | 54 | sm | _ | 6 | 2 | 38 |
| Sebeta | 48 | 54 | sm | m | 6 | 2 | 38 |
| Debre Sina | 48 | 62 | m | m | 8 | 8 | 30 |

### 5.3. C-banding

C-banding showed that there was variation in the amount and distribution of constitutive heterochromatic blocks in the chromosome complements of *T. splendens* in this study (Fig. 10 and Table 4). The largest number of autosomes containing C-positive blocks observed was 36, which was from Ziway and the smallest number observed was 4 from Asebe Teferi, Entoto and Sebeta (Table 4). Unfortunately, we couldn't get good metaphase spreads for C-banding from Bure and Debre Sina. In addition, we couldn't take good photos of C-banded chromosomes of *T. splendens* from the rest of the localities except Ziway and Masha (Fig. 10) for technical problems such as insensitivity of the film used to pick up small C-bands. Thus, C-band analysis was made under the microscope for most of the localities.

The C-bands were observed in the short arm (where short arm is available) and centromeric region. In addition, the Y-chromosome and in some cases the telomeric region of the X-chromosome showed C-positive bands. None of the autosomal metacentrics were C-positive from all the localities studied. Animals from Mugo and Masha showed almost similar C-bands. In both cases, only 12 of the autosomes (10 subtelocentrics and 2 acrocentrics) were C-positive while the rest of the autosomes were C-negative. The subtelocentrics had pericentromeric and short arm C-bands, whereas the acrocentrics had only pericentromeric bands. These types of heterochromatin localizations were common for all C-positive subtelocentric and acrocentric chromosomes in all the localities studied. The X-chromosome was C-negative in both Mugo and Masha. The Y-chromosome was fully heterochromatized in Masha, but no male specimen was studied for C-banding from Mugo.

Similarly, animals from Entoto and Sebeta showed similar patterns of C-banding. Only the two subtelocentric and two of the acrocentrics were C-positive autosomes. The only animals with C-positive X-chromosome were from these localities. Unfortunately, no male specimen was studied for C-banding from these localities.

*Tachyoryctes splendens* from Ziway had 36 C-positive autosomes (12 subtelocentrics and 24 acrocentrics), which was the maximum number observed in this study.

A female animal from Asebe Teferi had only 4 C-positive autosomes and all of which were subtelocentrics. The rest autosomes and the X-chromosome were C-negative.

The six subtelocentric autosomes and the Y-chromosome were C-positive in an animal from Alemaya whereas the rest chromosomes of the complement were C-negative.

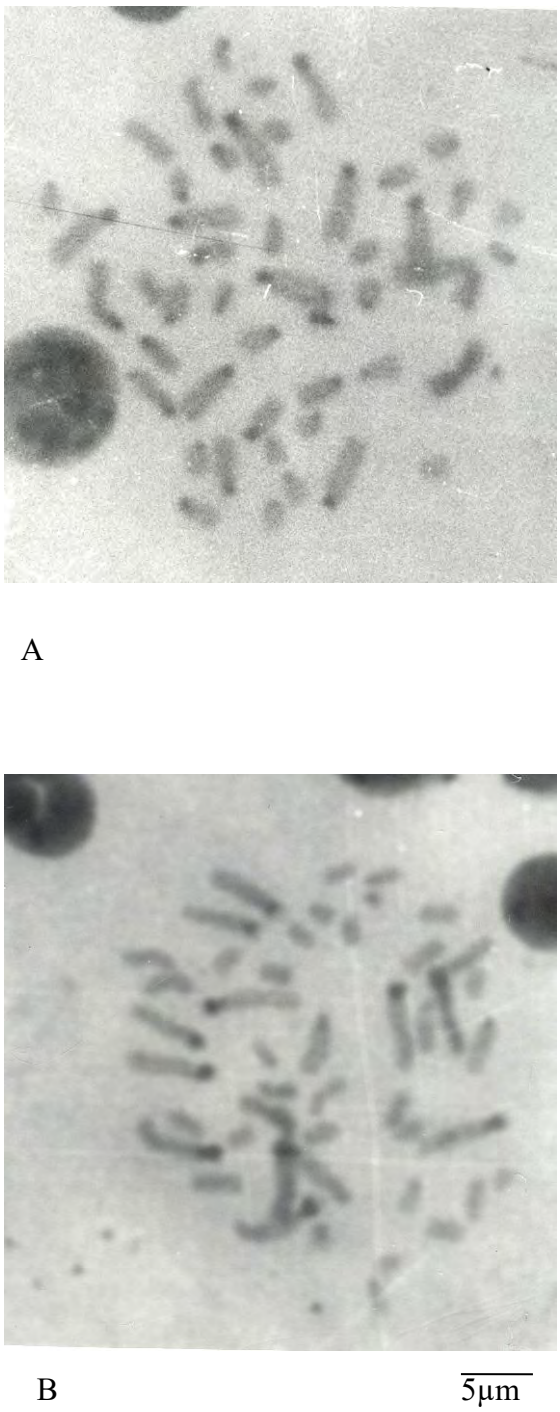



Fig. 10. C-banded chromosomes of *T. splendens* from: (A) Ziway male and (B) Masha male. Bar=5 µm.

Table 4. The number of C-positive chromosomes of each type (m, st, a*, X- & Y-) and the total number of C-positive autosomes in each locality.

| Chr. Type | | Locality | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mugo | | Masha | | Ziway | | Alemaya | | Asebe Teferi | | Entoto | | Sebeta | |
| | | No of chrs | C+ | No of chrs | C+ | No of chrs | C+ | No of chrs | C + | No of chrs | C + | No of chrs | C+ | No of chrs | C+ |
| Autosome | m | 10 | – | 10 | – | 6 | – | 6 | – | 6 | – | 6 | – | 6 | – |
| | st | 10 | 10 | 10 | 10 | 12 | 12 | 6 | 6 | 4 | 4 | 2 | 2 | 2 | 2 |
| | a | 26 | 2 | 26 | 2 | 28 | 24 | 34 | – | 36 | – | 38 | 2 | 38 | 2 |
| Sex | X-Y- | 2 | – | 1 | – | 1 | – | 1 | – | 2 | – | 2 | 2 | 2 | 2 |
| | | – | – | 1 | 1 | 1 | 1 | 1 | 1 | – | – | – | – | – | – |
| Total C+ Autosomes | | | 12 | | 12 | | 36 | | 6 | | 4 | | 4 | | 4 |

*a = acrocentric chromosome.

# 6. DISCUSSION

The chromosomes of *T. splendens* from nine different localities in Ethiopia were studied and six different karyotypic forms were found, of which only the karyotype of *T. splendens* from Alemaya is almost similar to the one described by Baskevich *et al*. (1993) for specimens from the Rift Vally near Koka area. The diploid chromosome number of *T. splendens* from all the nine localities studied is 48, which is in agreement with the results of other authors (Matthey, 1956; 1967 cited in Baskevich *et al*., 1993; Baskevich *et al*., 1993; Aniskin *et al*., 1997; Lavrenchenko *et al*., 1997). However, the autosomal fundamental number, the chromosome morphology, and the amount and distribution of heterochromatin are variable.

The karyotypes of the animals from Mugo, Masha and Bure are almost similar. They consist of 10 pairs of biarmed autosomes, of which 5 pairs are medium-sized metacentrics and the other 5 pairs are large subtelocentrics, 13 pairs of acrocentric autosomes, the largest metacentric X- and large (slightly smaller than the largest autosome) metacentric (perfect metacentric in Masha and Bure) Y-chromosome. In Mugo and Masha six pairs (5 pairs of subtelocentrics and a pair of subtelocentrics) of the autosomes are C-positives. The autosomal fundamental number is 66. This type of karyotypic form has not been reported before for this species, and especially the number of metacentric autosomes is a new finding of the present study.

Similarty in karyotypes is also found between Entoto and Sebeta specimens. This karyotype is comprised of 4 pairs of biarmed autosomes (3 pairs of metacentrics and a pair of subtelocentrics), 19 pairs of acrocentrics, C-positive X-, which is the largest in the complement, and large metacentric Y-chromosome in Sebeta (no male specimen from Entoto). The autosomal fundamental number is 54, which is the smallest NFa observed in this study. This karyotype is different from the rest, specially, in having the smallest number of biarmed autosomes and in the morphology of the X-chromosome, which is submetacentric. Of course, this type of X-chromosome (submetacentric) was reported before (Matthey, 1956; 1967 cited in Baskevich *et al*., 1993; Baskevich *et al*., 1993).

The karyotype of *T. splendens* from Ziway has the second largest number of biarmed autosomes observed in this study. Three pairs of the biarmed autosomes are metacentrics and the rest 6 pairs are subtelocentrics. The maximum number of C-positive autosomes is observed from a specimen

of this locality. The karyotypes of *T. splendens* from Alemaya and Asebe Teferi have a slight difference. The karyotype from Alemaya has 6 pairs of biarmed autosomes (3 pairs of metacentrics and 3 pairs of subtelocentrics), but that of Asebe Teferi has 5 pairs of biarmed autosomes (3 pairs of metacentrics and 2 pairs of subtelocentrics). The specimen from Debre Sina has a unique karyotype, especially, in the number of metacentric autosomes. The karyotype consists of 4 pairs of metacentric, 4 pairs of subtelocentric and 15 pairs of acrocentric autosomes, the largest metacentric X- and a large metacentric Y-chromosome.

In this study, though a total of 28 *T. splendens* specimens were collected (Table 1), only half of them were used for chromosome study (Appendix 2) due to lack of well spread metaphase chromosomes on slide preparations of the other specimens. The fact that the number of specimens from each locality is too small, due to the above reason, does not allow to infer about chromosomal variation within each population. However, there was no variation observed in this study between the two individuals from same localities in those cases where localities were represented with sample size of two specimens. Similarly, Baskevich *et al*. (1993) found no chromosomal variation within the population they studied. However, Aniskin *et al*. (1997) found intrapopulational variation in the number of C-positive autosomes and the autosomal fundamental number.

Most of the karyotypic forms of *T. splendens* described in this study are different from that of other authors (Matthey, 1956; 1967 cited in Baskevich *et al*., 1993; Baskevich *et al*., 1993; Aniskin *et al*., 1997; Lavrenchenko *et al*., 1997). As stated earlier, only the karyotype of the Alemaya specimen is almost similar to the one described by Baskevich *et al*. (1993). As cited in Baskevich *et al*. (1993), Matthey (1956) described (the first description) 48 chromosomal karyotype of *T. splendens* from unknown locality in Africa, which possess a great number of biarmed autosomes, mainly subtelocentric, and a large acrocentric Y-chromosome. The autosomal fundamental number of this karyotype was reported to be 83. As cited in Baskevich *et al*. (1993), Matthey (1967) also described the karyotype of *T. ruandae* Lonnberg and Gyldenstolpe 1925 from east Congo, for which the species rank is doubtful and it is considered as a subspecies form of *T. splendens* (Anderson and Jones, 1967; Sokolov, 1977). This subspecies, *T. s. ruandae*, has 48 chromosomes: two pairs of metacentric, 21 pairs of acrocentric autosomes,

the largest submetacentric X-chromosome and a large acrocentric Y-chromosome. The autosomal fundamental number was 51.

Baskevich *et al*. (1993) studied the karyotype of *T. splendens* from Ethiopia for the first time and they found that the karyotype consists of 48 chromosomes, of which 3 pairs are metacentric, 3 pairs subtelocentric, and 17 pairs acrocentric autosomes, the largest submetacentric X-chromosome and a large metacentric Y-chromosome. The autosomal fundamental number is 58. Using C-banding, they found that the sex chromosomes and almost all the autosomes are C-positives. Here, it differs from the karyotype of *T. splendens* from Alemaya, which has almost similar other karyotypic characteristics.

The karyotype of *T. splendens* from the Bale Mountains National Park described by Aniskin *et al*. (1997) consists of 48 chromosomes with the autosomal fundamental number ranging from 65-86. The karyotype comprises of 3 pairs of C-negative metacentric autosomes, 20 pairs of autosomes, which show morphological variations (a, st, and sm), the largest metacentric X-chromosome, and metacentric Y-chromosome. They found that the Y-chromosome is slightly larger than the largest autosome. In the present study, a similar result was found from Sebeta, but in other localities, it was the reverse, i.e., the Y-chromosomes is slightly smaller than the largest autosome. In the report of Aniskin *et al*. (1997) from the Bale Mountains National Park, the number of C-positive autosomes ranged from 13-34 and both of the sex chromosomes are C-positives. Lavrenchenko *et al*. (1997) also found 48 chromosomal karyotype of *T. splendens* with the autosomal fundamental number ranging from 68-86 at the same time from the Bale Mountains National Park. However, the number of observed C-positive autosomes ranged from 4-36 and that of the observed autosomal fundamental number ranged from 54-66 in this study.

As discussed above, the present work as well as the previous reports by other workers have shown the occurrence of chromosomal variation in *T. splendens* from different localities. This chromosome diversity among different populations of *T. splendens* can be attributed to the fossoriality of the animal (Baskevich *et al*., 1993). With fossoriality, demographic factors such as small deme size, law vagility, strong territoriality, etc., can enhance chromosomal variability and be involved in speciation and chromosome evolution (Nevo, 1979 cited in Baskevich *et al.,* 1993). Arnason (1972) also assumes that rodents, which are characterized by high reproduction (early

sexual maturity and many litters per year), restricted mobility and delimited niches, are predisposed to karyotypic variability. Ansikin *et al*. (1997) assume that the significant karyotypic variations found in *T. splendens* might be associated with the high lability of the species allowing rapid adaptation to various habitats.

Baskevich *et al*. (1993) suggested that the chromosomal variability observed in *T. splendens* were probably caused by pericentric inversions or heterochromatin variations. Indeed, some of the chromosomal variations in the present study are heterochromatic variations. This is evidenced by the fact that the differences in the number of subtelocentric chromosomes among *T. splendens* from different localities are due to the presence of additional short heterochromatic arms (as revealed by C-banding) on these chromosomes. This variation could be resulted either by amplification or loss of heterochromatin blocks.

The variation in the number of metacentric autosomes in *T. splendens* was most probably caused by pericentric inversions. Robertsonian fusion (one of the chromosomal rearrangements that cause formation of metacentrics) could not be the cause for this variation, since the diploid chromosome number (2n=48) is constant among all the karyotypes irrespective of the number of metacentric chromosomes. Heterochromatic amplification or loss could not also be the cause because all the metacentric chromosomes in this study are C-negatives.

In this study, five of the karyotypes have C-negative metacentric X-chromosomes, but two of the karyotypes, that of Entoto and Sebeta, have C-positive (in the telomeric region of the long arm) submetacentric X-chromosomes. This morphological feature of the X-chromosomes of Sebeta and Entoto could be due to the presence of a heterochromatic block in the telomeric region of the long arm, while it is absent in that of the other localities. This can also explain why the X-chromosomes from Entoto and Sebeta are longer than that of the other localities (Table 2). Baskevich *et al*. (1993) also found a similar X-chromosome (C-positive and submetacentric) from the Rift Valley and they explained that being submetacentric was due to the presence of a large heterochromatic block in the telomeric region of the long arm, which is in agreement with our result.

Baskevich *et al*. (1993) made two suggestions that the number of biarmed chromosomes decreases with the increase in aridity and the number of heterochromatic arms may exhibit an

altitudinal gradient. However, Aniskin *et al.* (1997) reported that their data did not confirm the second suggestion and they think that the changes in the number of chromosomes containing C-positive heterochromatic blocks were associated with other factors, the nature of which can be revealed by complex cytogenetic, morphological, physiological, and zoogeographical studies. The present study also did not confirm the previous suggestions of Baskevich *et al*. (1993) (Appendix 3).

Aniskin *et al*. (1997) suggested that migration of *T. splendens* to new ecologically different areas was accompanied by changes in the number of autosomes containing C-positive blocks and they provided the data on *T. splendens* from the Rift Valley (Baskevich *et al*., 1993) as a confirmation to their assumption. This implies that populations of *T. splendens* occupying ecologically different areas contain different number of C-positive autosomes. However, our finding shows that this is not always true. For example, *T. splendens* from Asebe Teferi and Entoto, which occupy ecologically different regions, have similar number of C-positive autosomes (Appendix 3).

The relationship between geographic barriers and chromosomal speciation has been vigorously debated (Gibson, 1984). He presented some examples showing three different cases. In the first case, karyotype difference can be found within a species across a geographical barrier. In another case, chromosomal differences can also be found within a species without the presence of an obvious geographical barrier. There is also a third case that geographic isolation may exist between populations without chromosomal differences. In any case, it is clear that geographic proximity and chromosomal diversity are not always correlated in *T. splendens.* For example, the distance between Entoto and Sebeta is about 30 kms and the specimens from these localitie have almost similar karyotypes, which correlates with the assumption on the basis of geographic proximity. On the other hand, Mugo and Bure are about 330 kms apart, and yet had almost similar karyotypes whereas Mugo and Ziway which are about 100 kms apart but differ in their karyotypes, which indicate that geographic proximity is not always correlated with chromosomal diversity.

# 7. CONCLUSIONS

The present study showed that there was no variation in the diploid chromosome number among *T. splendens* from different localities.

However, there was variation in the number of metacentric autosomes. There were also variations in the amount and distribution of heterochromatin, which in turn caused variations in autosomal fundamental number and in the morphology of the X-chromosome of *T. splendens* from different localities.

The present study showed that geographical proximity and chromosomal diversity are not always correlated in *T. splendens*.

This study did not confirm the earlier suggestions made by Baskevich *et al*.(1993) that the number of biarmed chromosomes decreases with the increase in aridity, and the number of heterochromatic arms may exhibit an altitudinal gradient.

Our data also did not confirm the other suggestion made by Aniskin *et al*. (1997), which states that migration of *T. splendens* to new ecologically different areas will be accompanied by changes in the number of autosomes containing C-positive blocks.

# 8. RECOMMENDATIONS

Based on the chromosome findings from the present study, we make the following recommendations:

- Further studies using other cytological techniques like G-banding may be helpful in understanding the origins of the variations in the number of metacentric autosomes revealed among *T. splendens* from different localities as well as other structural mutations which could not be revealed by the techniques used in the present study.

- Further chromosome study from other parts of the distribution range of the animal is needed to document all the karyotypic forms and to draw a valid conclusion about the chromosomes of the animal so that it can be used for clearing the taxonomy of this animal.

- Other studies, such as physiological and zoogeographical, could be helpful in revealing if there are any adaptive values associated with the number and size of C-positive heterochromatin blocks.

- Hybridization study may supply information if the karyological variations present among different populations could reflect genetic differentiation, which is useful information for taxonomic purpose.

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# APPENDICES

Appendix 1. Karyological data of *T. splendens*.

| Country | Locality | 2n | NFa | X– | Y– | m | st | a | C-positive | Reference | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Not given | | 48 | 83 | sm | a | large no of biarmed chrs | | | - | Matthey (1956) | Cited in Baskevich *et al*. (1993) |
| Congo | Eastern Congo | 48 | 51 | sm | a | 4 | 0 | 42 | - | Matthey (1967) | |
| Ethiopia | Rift Valley | 48 | 58 | sm | m | 6 | 6 | 34 | almost all | Baskevich *et al*. (1993) | |
| | BMNP* | 48 | 68-86 | m | m | 6 | varied | | 13-34 | Aniskin *et al*. (1997) | |
| | | 48 | 65-86 | m | m | 6 | varied | | - | Lavrenchencho *et al*. (1997) | |
| | Mugo | 48 | 66 | m | m | 10 | 10 | 26 | 12 | Our data | |
| | Masha | 48 | 66 | m | M | 10 | 10 | 26 | 12 | | |
| | Bure | 48 | 66 | m | M | 10 | 10 | 26 | - | | |
| | Ziway | 48 | 64 | m | m | 6 | 12 | 28 | 36 | | |
| | Alemaya | 48 | 58 | m | m | 6 | 6 | 34 | 6 | | |
| | Asebe Teferi | 48 | 56 | m | m | 6 | 4 | 36 | 4 | | |
| | Entoto | 48 | 54 | sm | m | 6 | 2 | 38 | 4 | | |
| | Sebeta | 48 | 54 | sm | m | 6 | 2 | 38 | 4 | | |
| | Debre Sina | 48 | 62 | m | m | 8 | 8 | 30 | - | | |

*BMNP= The Bale Mountains National Park

Appendix 2. Number of *T. splendens* studied and number of metaphase cells analyzed for chromosome length measurements and for C-banding.

| Locality | Sex | X- | | Y- | | Largest autosome | | C-band |
|---|---|---|---|---|---|---|---|---|
| | | Indivi-duals | cells | Indivi-duals | cells | Indivi-duals | cells | Indivi-duals |
| Mugo | M | 1 | 2 | 1 | 2 | 1 | 2 | – |
| | F | 1 | 7 | – | – | 1 | 8 | 1 |
| | Total | 2 | 9 | 1 | 2 | 2 | 10 | 1 |
| Masha | M | 1 | 8 | 1 | 8 | 1 | 8 | 1 |
| | F | – | – | – | – | – | – | – |
| | Total | 1 | 8 | 1 | 8 | 1 | 8 | 1 |
| Bure | M | 1 | 1 | 1 | 1 | 1 | 1 | – |
| | F | – | – | – | – | – | – | – |
| | Total | 1 | 1 | 1 | 1 | 1 | 1 | – |
| Ziway | M | 1 | 7 | 1 | 7 | 1 | 7 | 1 |
| | F | 1 | 4 | – | – | 1 | 4 | – |
| | Total | 2 | 11 | 1 | 7 | 2 | 11 | 1 |
| Alemaya | M | 1 | 5 | 1 | 5 | 1 | 5 | 1 |
| | F | 1 | 2 | – | – | 1 | 2 | 1 |
| | Total | 2 | 7 | 1 | 5 | 2 | 7 | 2 |
| Asebe Teferi | M | 1 | 4 | 1 | 4 | 1 | 4 | – |
| | F | 1 | 5 | – | – | 1 | 5 | 1 |
| | Total | 2 | 9 | 1 | 4 | 2 | 9 | 1 |
| Entoto | M | – | – | – | – | – | – | – |
| | F | 1 | 6 | 1 | – | 1 | 6 | 1 |
| | Total | 1 | 6 | 1 | – | 1 | 6 | 1 |
| Sebeta | M | 1 | 1 | 1 | 1 | 1 | 1 | – |
| | F | 1 | 5 | – | – | 1 | 5 | 1 |
| | Total | 2 | 6 | 1 | 1 | 2 | 6 | 1 |
| Debre Sina | M | 1 | 1 | 1 | 1 | 1 | 1 | – |
| | F | – | – | – | – | – | – | – |
| | Total | 1 | 1 | 1 | 1 | 1 | 1 | – |

Appendix 3. Climate data of the localities and the number of biarmed chromosomes and C-positive autosomes in each locality. (Source: Climate data from National Meteorological Services Authority of Ethiopia, and altitude from Sewnet Mengistu and Afework Bekele, 2003).

| Locality | Altitude (m) | Av. Ann. Rainfall (mm) | Av. Temp. (°C) | | No of biarmed chromosomes | No of C-positive Autosome |
|---|---|---|---|---|---|---|
| | | | Max. | Min. | | |
| Entoto | 2982 | 1160.9 | 18.6 | 8.9 | 10 | 4 |
| Sebeta | 2200 | 1076.3 | - | - | 10 | 4 |
| Ziway | 1665 | 668.4 | 27.7 | 14.3 | 20 | 36 |
| Debre Sina | 1900 | 1726.7 | - | - | 18 | - |
| Asbe Teferi | 1820 | 776.4 | 27.7 | 14.3 | 6 | 4 |
| Mugo | 2974 | - | - | - | 22 | 12 |
| Bure | 2670 | - | - | - | 22 | - |
| Alemaya | 2125 | 673.7 | 24.1 | 10.3 | 14 | 6 |
| Masha | 2325 | 1984.2 | 22.2 | 11.4 | 22 | 12 |

Appendix 4**.** Lengths in micrometer of X-chromosomes from different cells of *T. splendens* from different localities.

| | Mugo | Masha | Bure | Ziway | Alemaya | Asebe Teferi | Entoto | Sebeta | Debre Sina |
|---|---|---|---|---|---|---|---|---|---|
| | 5.9 | 6.4 | 6.4 | 6.4 | 7.7 | 5.0 | 8.6 | 10.0 | 5.0 |
| | 5.9 | 9.1 | | 6.4 | 6.4 | 7.3 | 11.4 | 8.2 | |
| | 7.3 | 6.8 | | 6.4 | 6.4 | 5.0 | 10.5 | 11.4 | |
| | 6.8 | 6.8 | | 9.1 | 6.4 | 8.2 | 9.5 | 11.4 | |
| | 7.3 | 7.7 | | 7.3 | 6.8 | 6.8 | 11.4 | 9.1 | |
| | 7.3 | 7.3 | | 8.2 | 6.8 | 6.8 | 8.6 | 8.6 | |
| | 6.6 | 7.3 | | 8.2 | 6.8 | 10.0 | | | |
| | 8.2 | 6.8 | | 6.8 | | 6.4 | | | |
| | 5.5 | | | 8.2 | | 6.4 | | | |
| | | | | 7.3 | | | | | |
| | | | | 6.8 | | | | | |
| Mean = | 6.7 | 7.3 | 6.4 | 7.4 | 6.8 | 6.9 | 10.0 | 9.8 | 5.0 |

Appendix 5**.** Lengths in micrometer of Y-chromosomes from different cells of *T. splendens* from different localities.

| | Mugo | Masha | Bure | Ziway | Alemaya | Asebe Teferi | Entoto | Sebeta | Debre Sina |
|---|---|---|---|---|---|---|---|---|---|
| | 3.2 | 4.5 | 3.6 | 4.5 | 3.6 | 3.6 | | 8.6 | 3.6 |
| | 3.6 | 5.9 | | 4.5 | 4.5 | 4.5 | | | |
| | | 3.2 | | 4.5 | 4.1 | 3.2 | | | |
| | | 4.1 | | 5.9 | 4.5 | 5.0 | | | |
| | | 4.5 | | 5.0 | 5.0 | | | | |
| | | 4.5 | | 5.5 | | | | | |
| | | 4.5 | | 5.5 | | | | | |
| | | 3.6 | | | | | | | |
| Mean = | 3.4 | 4.4 | 3.6 | 5.1 | 4.4 | 4.1 | | 8.6 | 3.6 |

Appendix 6**.** Lengths in micrometer of the largest autosomes from different cells of *T. splendens* from different localities.

| | Mugo | Masha | Bure | Ziway | Alemaya | Asebe Teferi | Entoto | Sebeta | Debre Sina |
|---|---|---|---|---|---|---|---|---|---|
| | 4.5 | 5.0 | 4.1 | 5.0 | 5.0 | 4.1 | 4.5 | 5.0 | 4.1 |
| | 5.0 | 5.9 | | 5.2 | 4.5 | 5.0 | 5.5 | 4.5 | |
| | 5.5 | 4.5 | | 4.8 | 4.5 | 3.6 | 5.5 | 6.8 | |
| | 6.1 | 3.9 | | 6.8 | 4.5 | 5.5 | 4.5 | 5.9 | |
| | 5.5 | 5.0 | | 5.0 | 4.5 | 4.5 | 5.5 | 4.5 | |
| | 4.1 | 5.0 | | 6.4 | 4.5 | 4.1 | 4.5 | 5.0 | |
| | 5.5 | 5.5 | | 6.8 | 4.5 | 5.9 | | | |
| | 5.2 | 4.1 | | 5.5 | | 4.1 | | | |
| | 6.4 | | | 5.5 | | 4.1 | | | |
| | 4.5 | | | 5.0 | | | | | |
| | | | | 5.0 | | | | | |
| Mean = | 5.2 | 4.9 | 4.1 | 5.5 | 4.6 | 4.5 | 5.0 | 5.3 | 4.1 |