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KARYOTYPE ANALYSIS OF THE COMMON MOLE RAT (*TACHYORYCTES SPLENDENS*) FROM DIFFERENT LOCALITIES IN ETHIOPIA

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ABSTRACT: In the present study, karyotypes of the east African mole rat, *Tachyoryctes splendens*, specimens from nine localities in Ethiopia namely; Bure, Chiro, Debre Sina, Entoto, Haramaya, Masha, Mugo, Sebeta and Ziway, were analyzed using conventional chromosome preparation method from the bone marrow cells. 2n=48 chromosomes were counted for all the specimens of all the nine localities. The chromosomes fall into metacentric, submetacentric, subtelocentric and telocentric classes, with the latter being the most frequent type. In all the cases, the X chromosome was the largest in the complement and it was metacentric, except for Entoto and Sebeta specimens, in which case it was submetacentric. The Y chromosome was smaller than the X chromosome and it was also metacentric in all the karyotypes except in Debre Sina where it was submetacentric. In all the karyotypes, small autosomal metacentric chromosomes were present, but their number varied as three, four and five pairs in different karyotypes. Clear submetacentric autosomal chromosomes were observed in the karyotypes of Masha and Mugo specimens only. Autosomal fundamental number ranged from 52 to 66. Generally, karyotypic similarity corresponded with population geographic proximity. In total, six different karyotypic forms were recognized which can be grouped as Masha-Mugo, Bure, Debre Sina, Entoto-Sebeta, Ziway, and Haramaya-Chiro karyotypes. The presence of different numbers of metacentric autosomal chromosomes observed in different karyotypes, unaccompanied by a change in diploid chromosome number, could probably be due to pericentric inversions rather than due to Robertsonian translocations.

Key words/phrases: Chromosome, Ethiopia, Karyotype, *Tachyoryctes splendens*.

INTRODUCTION

The east African mole rat (*Tachyoryctes splendens*) belongs to the mammalian order Rodentia. The exact number of species belonging to the genus *Tachyoryctes* Ruppell 1835 is not clearly known (Nowak, 1991), and a varying number of species have been reported by different authors. Some authors (Anderson and Jones, 1967; Sokolov, 1977; Honacki *et al*., 1982; Corbet and Hill, 1991) consider the number of species to be two by lumping all known forms, and recognizing *T. macrocephalus*, and *T. splendens*. On the other hand, Ellerman (1941) considered *T. splendens* to be one of the 14

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species of this genus. Furthermore, this genus has no fixed position with regard to taxonomic family. Ellerman (1941) placed it in the family Muridae, while Allen (1939), Anderson and Jones (1967), Sokolov (1977), Honacki *et al*. (1982) placed the genus in the family Rhizomyidae.

Tachyoryctes splendens is a solitary, fossorial rodent, which is distributed over the uplands of northeastern Africa, including Ethiopia, parts of Somalia, Kenya, Rwanda, Burundi, Uganda and northern Tanzania, and extends westward as far as eastern Zaire (Jarvis and Sale, 1971; Nowak, 1991). In Ethiopia, the species is mostly found between 1300 and 3900 meters above sea level (Yalden *et al*., 1976).

Chromosome data are useful for taxonomic purposes and for possible clues to the evolution of related groups of species (Garber, 1979). The existence of a large number of karyotypic variations among species is considered as evidence that there is association between evolutionary processes and karyotypic changes (Fredga, 1977). Most groups of animals, even the most closely related ones, 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, 1975). Bush *et al*. (1977) 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).

Though chromosome number is generally constant for a species, variable number of chromosomes has been reported in some mammalian species, which was attributed mainly to Robertsonian translocation (Wahrman *et al*., 1969; Searle *et al*., 1990; Nevo *et al*., 1994). However, so far, the diploid chromosome number of *T. splendens* has been reported to be 2n=48 (Baskevich *et al*., 1993; Aniskin *et al*., 1997; Lavrenchenko *et al*., 1997).

The number of chromosome arms shows variation in some rodent species. Similarly, the number of chromosome arms of *T. splendens* also showed variation. Autosomal fundamental numbers (NFa) in the range of 58 and 86 have been reported for this species (Baskevich *et al*., 1993; Aniskin *et al*., 1997; Lavrenchenko *et al*., 1997).

Studying the morphology of chromosomes and classifying them accordingly helps to reach at an understanding of how new karyotype evolution has proceeded and to find correlations with the evolution of other systematic characteristics such as anatomical, biochemical and behavioural (Levan *et*

al., 1964). The position of the centromere is one of the morphological features of mitotic metaphase chromosomes. It is the most useful landmark, which is characterized by great constancy (Levan *et al*., 1964).

The present investigation was aimed at studying number and morphology of the chromosomes of *T. splendens* from different localities of Ethiopia as an attempt towards a better understanding of the taxonomy of the genus.

MATERIALS AND METHODS

Sampling locality

Specimens were collected from nine localities in Ethiopia (Fig. 1 and Table 1). The latitudinal and longitudinal coordinates and altitude of each locality, and the number of specimens studied are presented in Table 1.

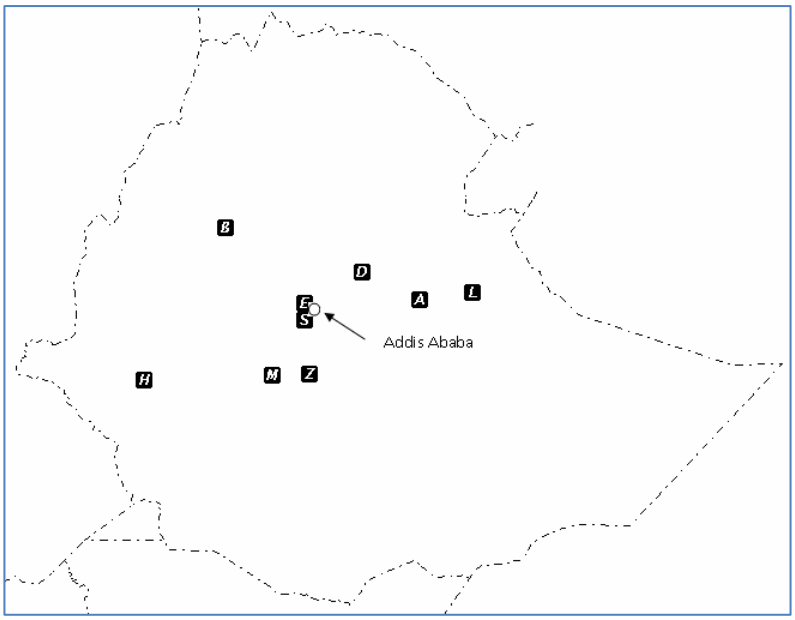

Fig. 1. Map of Ethiopia showing collection sites of *T. splendens* specimens: (A) Chiro; (B) Bure; (D) Debre Sina; (E) Entoto; (H) Masha; (L) Haramaya; (M) Mugo; (S) Sebeta and (Z) Ziway.

The localities were selected on the basis of geographic location to represent distribution range of the species in the country and the presence of the species in the localities as reported in an earlier work (Sewnet Mengistu and Afework Bekele, 2003).

Table 1. Geographic location and altitude of localities of specimen collection and the number of *T. splendens* specimens analyzed.

Locality	Latitude	Longitude	Altitude (m)	Male	Female	Total
Bure	10°40'30'N	37°04'20"E	2670	1	-	1
Chiro	9°04'30"N	40°51'51"E	1820	1	1	2
Debre Sina	9°50'00"N	39°16'25"E	1900	1	-	1
Entoto	9°02'30"N	38°35'50"E	2982	-	1	1
Haramaya	9°27'N	42°01'E	2125	1	1	2
Masha	7°43'N	35°28'E	2325	1	-	1
Mugo	7°50'18"N	37°59'06"E	2974	1	1	2
Sebeta	8°55'N	38°38'E	2200	1	1	2
Ziway	7°52'25"N	38°43'44"E	1665	1	1	2
Total				8	6	14

Specimen 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. Macabee gopher traps (MGT model) were used. Traps were inserted into the burrow and tied to a wooden stick partly entrenched in the ground to prevent the traps from being taken inside the burrow. Live-trapped mole rats from Entoto and Sebeta were transported to the Genetics Laboratory at Science Faculty, Addis Ababa University, for chromosome preparation. In the case of the other localities, chromosome preparations were made in the nearby schools or agricultural bureaus.

Metaphase chromosome preparation

Somatic metaphase chromosome preparations were made from bone marrow cells following Baker (1970) with some modification as follows:

Individual mole rats were weighed using a hand-balance, injected with 1ml colchicine (0.05%) per 100 gm body weight, and left in a rat cage for one and half hours. The animal was then killed by over etherization; the femur bone was dissected, cleared of muscle tissues and crushed in a Petri dish containing 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 5 minutes, the supernatant was discarded, and the pellet was suspended in about 3 ml freshly prepared fixative (3 parts methanol and 1 part acetic acid, V/V). After a minimum of 10 minutes of fixation at room temperature, the suspension was centrifuged at 1000 rpm for 5 minutes. After 3 more rounds of suspension in fixative followed by centrifugation, the pellet was re-suspended in 0.5 ml of fixative for slide preparation. Slides were prepared by splashing 2 or 3 drops of the cell suspension onto a clean slide from a height of 0.5m. The slides were

then air-dried and stored. Air-dried slides were stained using the conventional Giemsa staining technique.

Karyotype analysis

Photomicrographs of good metaphase chromosome spreads were taken using NIKON microscope fitted with NIKON FX-35DX camera with a magnification of x100 objective. Chromosomes were described and characterized using photomicrographs as well as direct observation under the microscope. The karyotypes were constructed from enlarged photomicrographs by arranging putative homologous chromosomes into pairs using centromeric position and chromosome size as criteria. The suggestion of Levan *et al*. (1964) was followed in defining the centromeric position of the chromosomes with some modifications such that "M" and "m" are referred to as metacentric, "sm" as submetacentric, "st" as subtelocentric and "t" and "T" as telocentric.

RESULTS

Chromosome number

No difference was observed 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. 2-7 and Table 2). However, some differences were observed regarding the number of bi-armed autosomal chromosomes present and, to some extent, in the morphology of sex chromosomes.

Karyotype description

In describing the karyotypes those populations with similar karyotypes have been considered together.

Tachyoryctes splendens from Mugo and Masha showed virtually similar karyotypes (Fig. 2). The autosomal complements of *T. splendens* from these two localities consisted of 5 pairs of small metacentric (m), 5 pairs of submetacentric (sm) and 13 pairs of telocentric (t) chromosomes. The five pairs of metacentric chromosomes were more or less of equal size. The five pairs of submetacentric autosomal chromosomes were relatively larger than both the metacentric and telocentric chromosomes. The autosomal fundamental number of *T. splendens* from these two localities was 66. The X chromosome was the largest chromosome in the complement and it was metacentric. The Y chromosome was also metacentric, but it was smaller than the X chromosome.

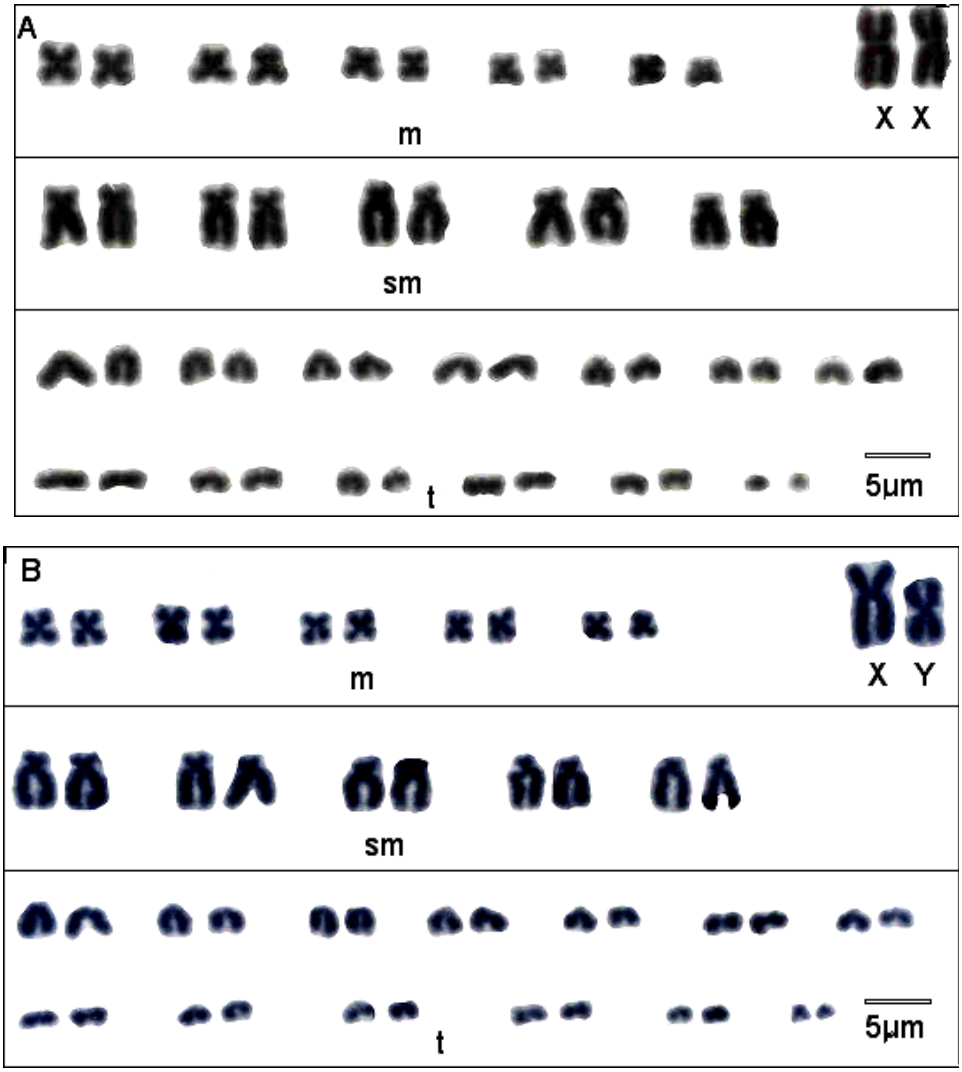

Fig. 2. Karyotypes of *T. splendens* from Mugo and Masha: (A) Mugo female and (B) Masha male. (m = metacentric, sm = submetacentric and t = telocentric). Bar = 5µm

The karyotype of Bure specimen of *T. splendens* (Fig. 3) was largely similar to that of Masha and Mugo specimens, particularly, in the presence of 5 pairs of small metacentric autosomal chromosomes, but the autosomal fundamental number was 56, instead of 66. As in the Masha and Mugo karyotypes, the X and Y chromosomes of the Bure specimen were metacentric, with Y being smaller than X.

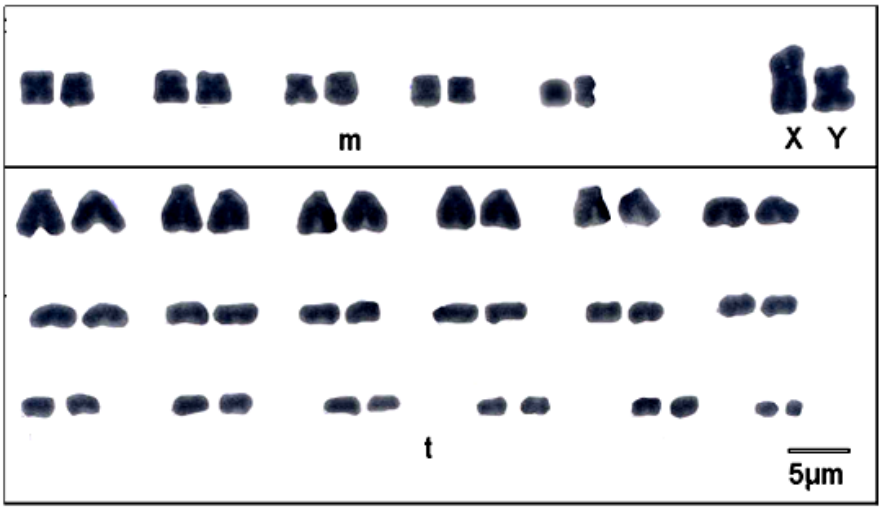

Fig. 3. Karyotype of male *T. splendens* from Bure (m = metacentric and t = telocentric). Bar = 5µm

Tachyoryctes splendens from Debre Sina had a karyotype, which was composed of 4 pairs of metacentric, 3 pairs of subtelocentric and 16 pairs of telocentric autosomal chromosomes, and a metacentric X and a submetacentric Y chromosome (Fig. 4). The four pairs of autosomal metacentric chromosomes had similar size. Among the telocentric autosomes, three pairs were relatively larger than the rest. The X chromosome was the largest in the complement and the Y chromosome was about half the size of the X chromosome. The autosomal fundamental number was 60.

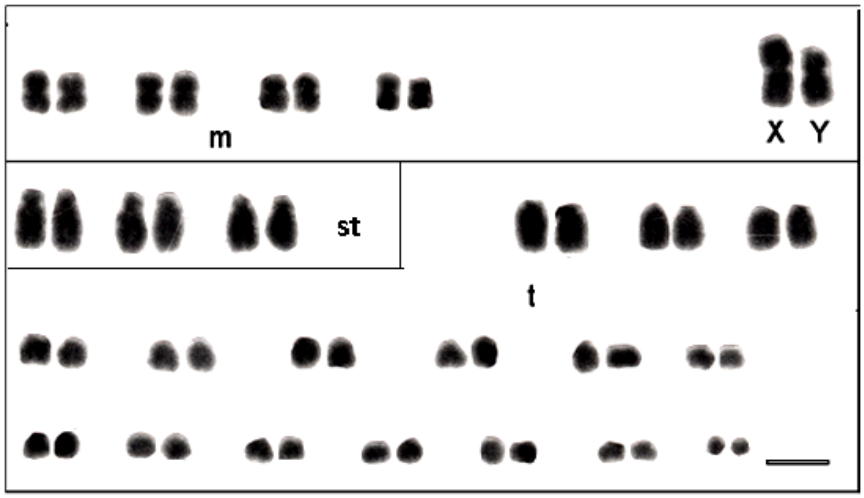

Fig. 4. Karyotype of male *T. splendens* from Debre Sina (m = metacentric, st = subtelocentric and t = telocentric). Bar = 5µm

Tachyoryctes splendens specimens from Entoto and Sebeta had identical karyotypes (Fig. 5). The karyotype was comprised of 3 pairs of small autosomal metacentrics, with one of the pairs being slightly larger, 20 pairs of telocentric autosomes, and a pair of sex chromosomes. The X chromosome was the largest in the complement and it was submetacentric, whereas the Y chromosome, as observed in Sebeta specimen, was metacentric and smaller than the X chromosome. The autosomal fundamental number was 52 for specimens from both localities.

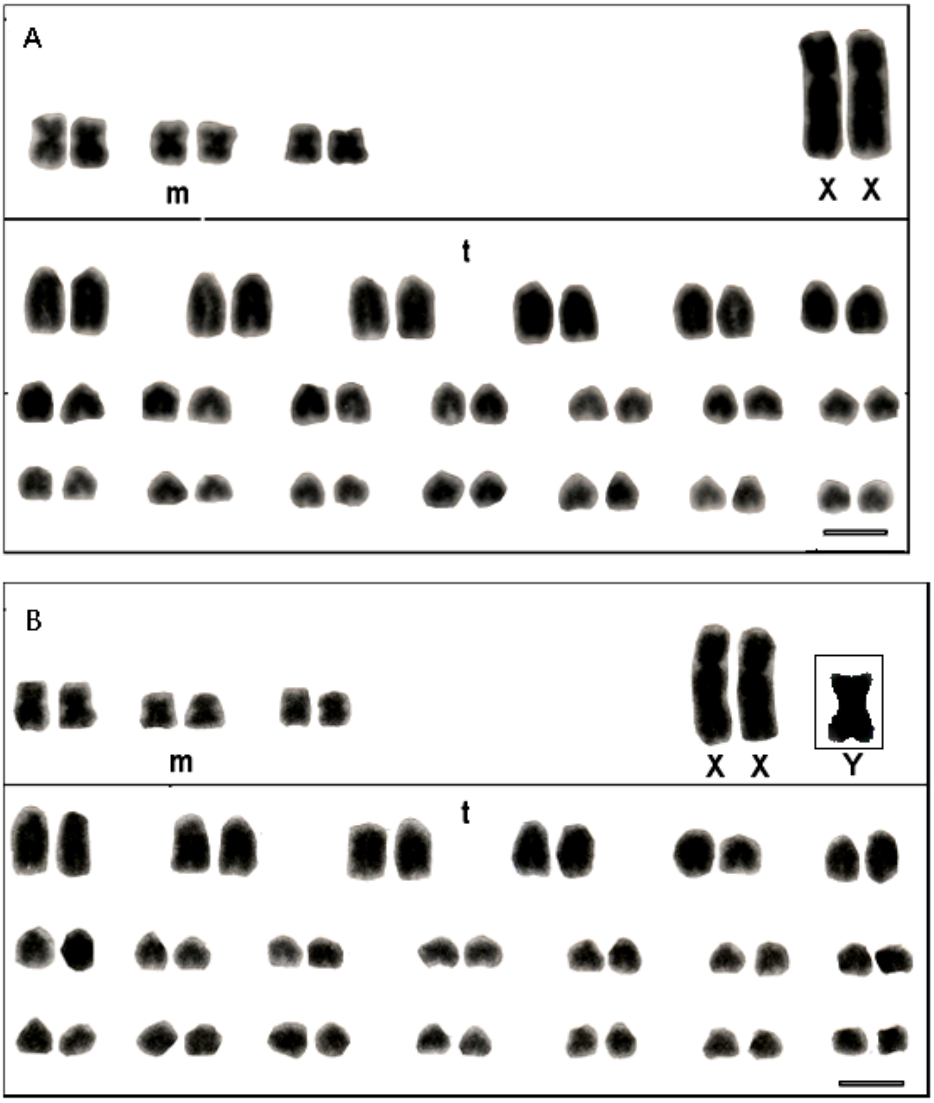

Fig. 5. Karyotypes of *T. splendens* from Entoto and Sebeta: (A) Entoto female and (B) Sebeta female (Y is inserted from another complement, male) (m = metacentric and t = telocentric). Bar = 5µm

The karyotype of *T. splendens* from Ziway (Fig. 6) was similar to the karyotypes of Entoto and Sebeta specimens in having three pairs of small autosomal metacentrics, one pair of which was relatively larger than the other two pairs. However, unlike the X chromosomes from Entoto and Sebeta specimens, the X chromosome of Ziway specimens was a metacentric chromosome. The autosomal fundamental number for Ziway specimens was also 52.

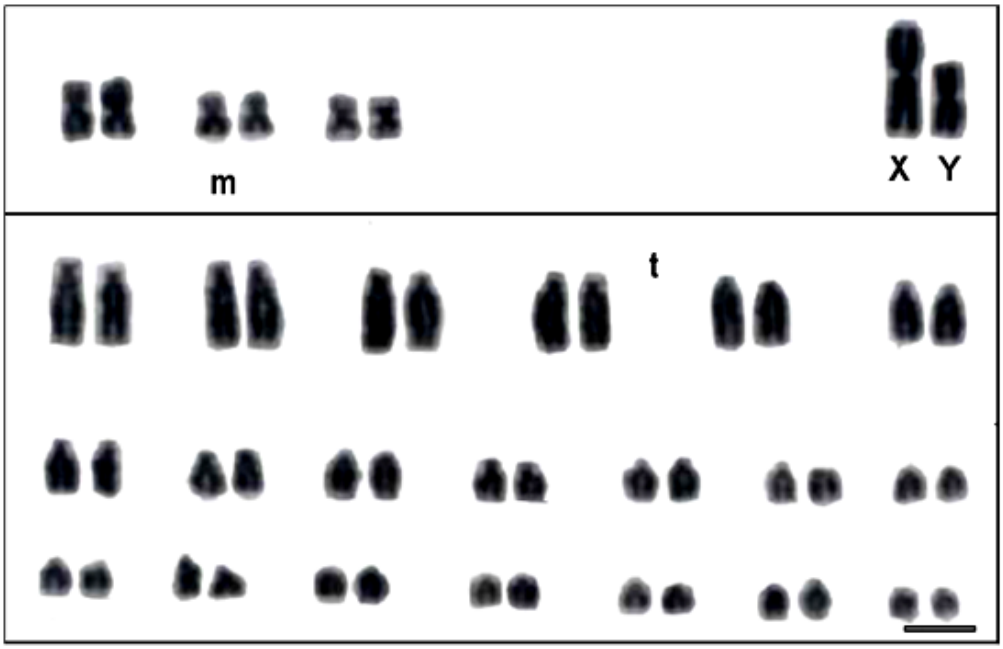

Fig. 6. Karyotype of male *T. splendens* from Ziway (m = metacentric and t = telocentric). Bar = 5µm

The karyotypes of *T. splendens* from Haramaya and Chiro (Fig. 7) were identical. The autosomal chromosomes consisted of 3 pairs of metacentrics, and 20 pairs of telocentrics. As in other karyotypes, six pairs of the telocentric chromosomes were relatively larger than the rest of the telocentrics as well as the metacentric chromosomes. The X chromosome was metacentric and it was the largest in the complement, whereas the Y chromosome was a metacentric of about half the size of the X chromosome. The autosomal fundamental number was 52.

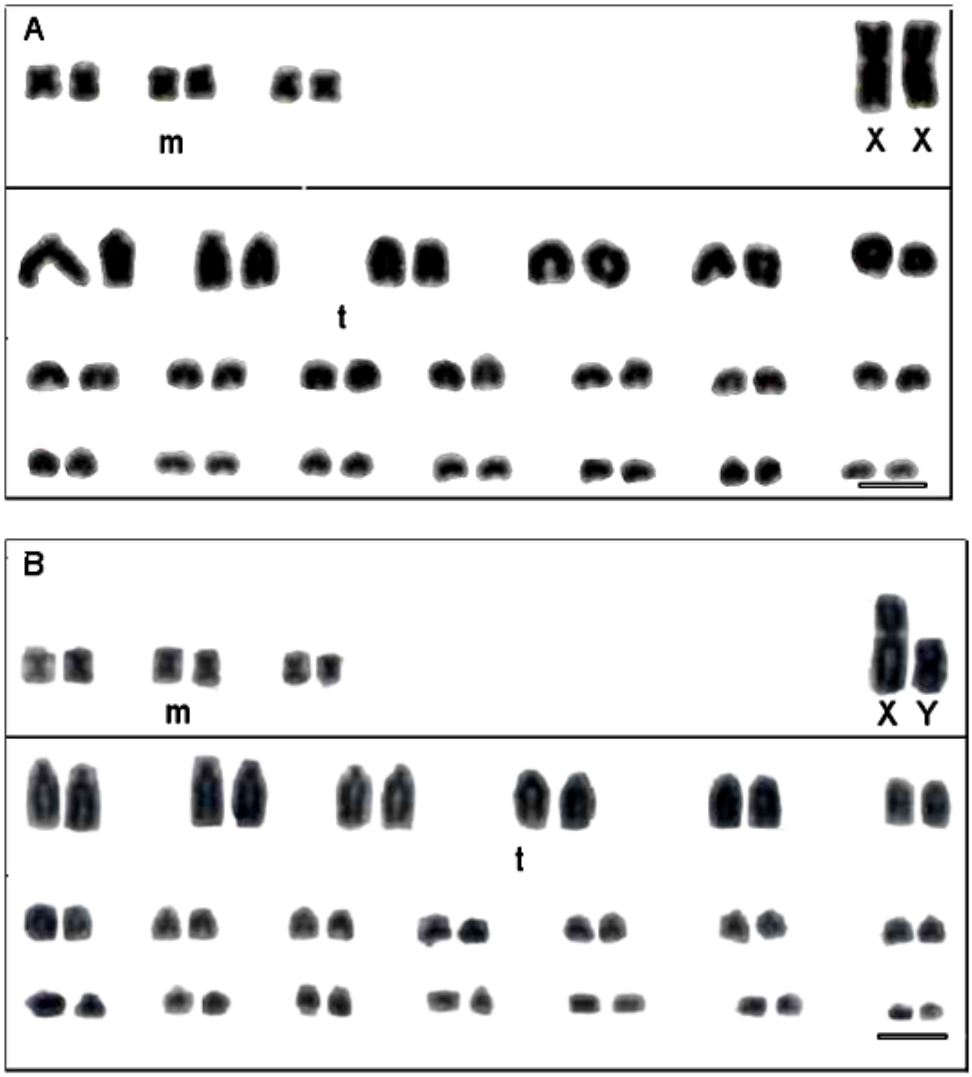

Fig. 7. Karyotypes of *T. splendens* from Chiro and Haramaya: (A) Chiro female and (B) Haramaya male (m = metacentric and t = telocentric). Bar = 5µm

Table 2. Karyological data of *T. splendens* from different localities in Ethiopia showing diploid chromosome number (2n), autosomal fundamental number (NFa), centromeric positions of sex chromosomes (X and Y) and number of autosomes with m (metacentric), sm (submetacentric), st (subtelocentric) and t (telocentric) centromeric positions.

			Sex chromosomes		**Autosomal chromosomes**			
Locality	**2n**	**Nfa**	**X**	**Y**	**m**	**sm**	**st**	**t**
Bure	48	56	m	M	10	-	-	36
Chiro	48	52	m	m	6	-	-	40
Debre Sina	48	60	m	sm	8	-	6	32
Entoto	48	52	sm	_	6	-	-	40
Haramaya	48	52	m	m	6	-	-	40
Masha	48	66	m	M	10	10	-	26
Mugo	48	66	m	M	10	10	-	26
Sebeta	48	52	sm	m	6	-	-	40
Ziway	48	52	m	m	6	-	-	40

DISCUSSION

In the present study, the karyotypes of *T. splendens* from nine localities in Ethiopia were analyzed. The result showed constancy in chromosome number but variation was observed in some other karyotypic features. The diploid chromosome number (2n) of *T. splendens* from all the nine localities studied was 48, which is in agreement with the results of other authors (Baskevich *et al*., 1993; Aniskin *et al*., 1997; Lavrenchenko *et al*., 1997). However, autosomal fundamental number (NFa) and chromosome morphology observed in the present study differed from what were reported by the earlier authors.

The karyotypes of the mole rats from Mugo and Masha were almost identical. A karyotype similar to this one has not been reported before for this species, and especially the presence of five pairs of metacentric autosomes is a new finding of the present study. The same is true for the karyotype of the Bure specimen, which was very closely similar to that of Masha and Mugo specimens.

Identity in karyotypes was also seen between the Entoto and Sebeta specimens. The presence of submetacentric X chromosome distinguishes these karyotypes from all others described in the present study. This type of X chromosome was reported before for this species (Baskevich *et al*., 1993).

The Ziway karyotype was similar to the karyotypes of Entoto and Sebeta except that the X chromosome of Ziway specimens was more symmetrical (metacentric) than that of Entoto and Sebeta, which was submetacentric.

The karyotypes of Haramaya and Chiro specimens were identical. These karyotypes were similar to that of specimens of Entoto, Sebeta and Ziway in having three pairs of metacentric autosomal chromosomes, but in the former all the three pairs were of similar size whereas in the latter group one pair was larger than the other two pairs.

The karyotype of the Debre Sina specimen differed from all the other described in the present study by being the only one to contain four pairs of metacentric autosomal chromosomes whereas the other karyotypes contained either five or three pairs of such chromosomes. In addition, in the present study, this karyotype was the only one to contain subtelocentric autosomal chromosomes and a submetacentric Y chromosome.

All the karyotypic forms of *T. splendens* described in this study were different from that reported by other authors (Baskevich *et al*., 1993; Aniskin *et al*., 1997; Lavrenchenko *et al*., 1997).

Baskevich *et al*. (1993) studied the karyotype of *T. splendens* from Ethiopia, upper part of the Awash valley near Koka area, for the first time and they found that it consisted of 48 chromosomes (6 pairs of bi-armed and 17 pairs of acrocentric autosomes, the largest submetacentric X, and a large metacentric Y chromosome). This karyotype does not correspond to any of the karyotypes described in our study.

Lavrenchenko *et al*. (1997) found the karyotype of *T. splendens* also from Ethiopia, the Bale Mountains National Park, with NFa ranging from 68 to 86. Aniskin *et al*. (1997) also found the karyotype of *T. splendens* from the same area with NFa ranging from 65 to 86. However, our result is different in that the observed NFa ranged from 52 to 66.

In the present study, since the number of specimens studied from each locality was too small, it would not allow to detect if there was any intrapopulation chromosomal polymorphism of *T. splendens* within each locality. Nevertheless, there was no chromosomal variation observed in this study between the two individuals from same locality in those cases where localities were represented by two specimens. A similar result was reported by Baskevich *et al*. (1993); chromosome variability was not revealed among the four *T. splendens* individuals studied. To the contrary, Aniskin *et al*. (1997) found intrapopulation variations in autosomal fundamental number within each three local populations studied from the Bale Mountains National Park, Ethiopia.

As could be seen from the foregoing discussions, the karyotypes described

in the present study may be grouped into six types as follows: (1) Masha-Mugo karyotype, (2) Bure karyotype, (3) Debre Sina karyotype, (4) Entoto-Sebeta karyotype, (5) Ziway karyotype, and (6) Haramaya-Chiro karyotype. However, it is noticeable that the difference between karyotype 4 and 5 is a minor one which is based only on the degree of asymmetry of the X chromosome.

As discussed above, the present work as well as the previous reports by other authors has shown the occurrence of chromosomal variation among *T. splendens* populations 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, low vagility, strong territoriality, etc., can enhance chromosomal differentiation among populations and play a role in speciation and chromosome evolution (Nevo, 1979; Toloza *et al*., 2004). Arnason (1972) also assumes that rodents, which are characterized by high rate of reproduction, restricted mobility and delimited niches, are predisposed to karyotypic variability.

Baskevich *et al*. (1993) suggested that the chromosomal variability observed in *T. splendens* were probably caused by pericentric inversions or heterochromatin variations. The variation in the number of metacentric autosomes in *T. splendens* was most probably caused by pericentric inversions. Robertsonian fusion could not be the cause for this variation, since the diploid chromosome number was constant among all the karyotypes irrespective of the number of metacentric chromosomes they possessed, unless it is assumed that the small metacentric product of the translocation also survived, which is not usually the case.

The relationship between geographic barriers and chromosomal speciation has been vigorously debated (Gibson, 1984). Generally, it has been found that populations with close geographic proximity or without apparent geographic barrier between them have more similar or identical karyotypes. This general trend was observed in the present study. However, as observed in the present study, Mugo is about 100 km and 250 km away from Ziway and Masha, respectively, but there was more karyotypic similarity between Mugo and Masha rather than between Mugo and Ziway populations. Some sort of ecological or geographical barrier could isolate the Mugo and Ziway populations, rather than distance.

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

Ethiopia. However, there was variation in the number of metacentric autosomes, in the autosomal fundamental numbers and in the morphology of the X chromosome. Previous reports by other authors have also shown the occurrence of chromosomal variations among *T. splendens* populations from different localities. Thus, the existence of such chromosomal variations in *T. splendens* agrees with the great morphological diversity in this taxon. This implies that revision could be needed on the taxonomy of *Tachyoryctes*. But, our result is not enough to make such a revision; further study is needed.

In order to generate useful information about the extent of chromosomal variation that exists in this species, the nature of chromosome structural changes responsible for chromosomal variation between populations and to determine to what extent the karyotypic differentiations affect breeding between different populations, we recommend that (1) more populations covering the distributional range of the species be chromosomally studied; (2) chromosome study techniques such as G-banding and molecular cytogenetics be employed and (3) controlled crosses between populations having different karyotypes be carried out.

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