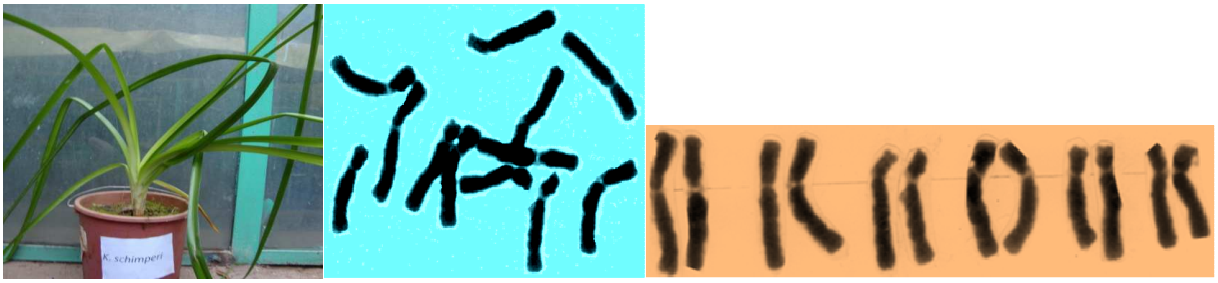
Addis Ababa University

School of Graduate Studies

Biology Department

Chromosome Study in Five Endemic *Kniphofia* Species (Asphodelaceae) of Ethiopia



By
Fekadu Gadissa

A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial fullfilment of the requirement for the Degree of Masters of Science in Biology (Applied Genetics Stream).

November 2009
Addis Ababa

Addis Ababa University

School of Graduate Studies

Biology Department

Chromosome Study in Five Endemic *Kniphofia* Species (Asphodelaceae) of Ethiopia

A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial fullfilment of the requirement for the Degree of Masters of Science in Biology (Applied Genetics Stream).

By

Fekadu Gadissa

Advisors:-

1. Kifle Dagne (PhD)
2. Ensermu Kelbessa (Prof.)

September 2009
Addis Ababa

Table of Contents

Page

LIST OF FIGURES----III

LIST OF TABLES----IV

LIST OF APPENDICES----V

ACKNOWLEDGEMENTS----VI

ABSTRACT----VII

1. **Introduction**----1

1.1. The Family Asphodelaceae----1

1.2. The genus Kniphofia----2

1.2.1 Botany and distribution of the genus *Kniphofia*----3

1.2.1. Reproduction----4

1.2.2. Economic Importance----4

1.2.3. Conservation----5

1.3. The genus *Kniphofia* Moench in Ethiopia----5

1.3.1. *Kniphofia foliosa* Hochst.----6

1.3.2. *Kniphofia hildebrandtii* Cufod.----6

1.3.3. *Kniphofia insignis* Rendle.----7

1.3.4. *Kniphofia isoetifolia* Hochst.----7

1.3.5. *Kniphofia schimperi* Baker.----8

1.4. Cytogenetics and its significance in plant systematic----8

1.4.1 Karyotype----9

1.4.1.1 Chromosome number----10

1.4.1.2 Chromosome size----11

1.4.1.3 Chromosome morphology----11

1.4.1.3.1. Centromeric position ----12

1.4.1.3.2. Secondary constriction and satellites----12

1.4.1.4 Karyotype symmetry----13

1.4.2 Karyotype Evolution----13

1.4.2.1 Changes in chromosome number----14

1.4.2.2 Change in chromosome gross morphology----------14
1.5. B-**Chromosomes----------16**
1.5.1 Distribution of B chromosomes----------17
1.5.2 Origin and evolution of B chromosomes----------17
1.5.3 The role of B chromosomes----------18
1.6. **Karyotype studies in *Kniphofia* Moench ----------18**

2. Objectives of the Study----------20
2.1. General Objective----------20
2.2. Specific Objectives----------20

3. Materials and Methods----------21
3.1. Plant materials----------21
3.2. Pretreatment of roots for chromosome preparation----------22
3.3. Air-dry slide preparation----------23

4. Results----------24
4.1. *Kniphofia foliosa*----------24
4.2. *Kniphofia schimperi*----------26
4.3. *Kniphofia insignis*----------28
4.4. *Kniphofia hildebrandtii*----------32
4.5. *Kniphofia isoetifolia*----------33

5. Discussion----------35

6. Conclusion and Recommendations----------40
6.1. Conclusion----------40
6.2. Recommendations----------41
7. References----------42

List of figures

Page

Figure 1 Picture of *Kniphofia* samples used in the present study: *K. foliosa* (A), *K. schimperi* (B), *K. hildebrandtii* (C), *K. insignis* (D), *K. isoetifolia* (E)----------22

Figure 2 Metaphase chromosome spread (A) and karyotype constructed (B) of *K. foliosa,* Bar=10µm----------25

Figure 3 Chromosome satellites (arrow) of *K. foliosa*. Bar = 10µm----------26

Figure 4 Mitotic anaphase stage showing normal disjunction of the B-chromosome (arrows) of *K. foliosa*. Bar = 10µm----------26

Figure 5 Metaphase chromosome spread (A), karyotype constructed (B) and chromosome satellites (C) for *K. schimperi*. Bar- 10µm----------27

Figure 6 Metaphase chromosome spread (A) and karyotype (B) of *K. insignis*. Bar =10µm----30

Figure 7 Chromosome of *K. insignis* showing secondary constriction. A. Partial complement with long arrows indicating secondary constriction, short arrows the satellite and arrow head showing region lying between two constrictions. B. Less condensed chromosomes showing a promenant secondary constriction (arrow). Bar-10µm----------31

Figure 8 Metaphase chromosome spread (A), karyotype (B) of *K. hildebrandtii*. Bar = 10µm--32----------32

Figure 9 Chromosome satellites of *K. hildebrandtii*. Bar = 10µm----------33

Figure 10 Metaphase chromosome spread (A) and karyotype (B) of *K. isoetifolia.* Bar = 10µm----------34

Figure 11 Chromosome spread of *K. isoetifolia* showing satellites (arrows)----------34

Figure 12 Speculation about the origin of satellite chromosome and reduction of chromosome number in *K. insignis*.----------37

List of tables

Page

Table 1 Species used in the present study and their collection site----------21
Table 2 Summary of chromosome counts of the studied species----------24
Table 3 Mean arm ratios of the chromosomes and designation of centeomeric positions. (Letters within brackets represent chromosome type)----------28
Table 4 Summary of the average chromosome length of individual pairs and total chromosome length per haploid genome of the studied species in µm----------29
Table 5 Average symmetry/asymmetry of chromosome complements of the five *Kniphofia* species based on the ratio of the total length of all the long arms to the total length of all the short arms of 2n chromosomes----------33

List of Appendices

Page

Appendix 1 Chromosome measurements in (cm), arm ratios and position of centromers in *Kniphofia foliosa*----------49

Appendix 2 Chromosome measurements in (cm), arm ratios and position of centromers in *Kniphofia schimperi*----------50

Appendix 3 Chromosome measurements in (cm), arm ratios and position of centromers in *Kniphofia hildebrandtii*----------51

Appendix 4 Chromosome measurements in (cm), arm ratios and position of centromers in *Kniphofia isoetifolia*----------52

Appendix 5 Chromosome measurements in (cm), arm ratios and position of centromers in *Kniphofia insignis*----------53

Acknowledgements

I would like to express my sincere appreciation and thanks to my research advisor Dr. Kifle Dagne for his very precious time, patience and unreserved guidance throughout the study time and provision of necessary materials for the research. My sincere thanks also go to my advisor Prof. Ensermu Kelbessa for his kind help in identifying the plant material, advice, comments on the thesis and valuable support in the collection and taxonomic identification of the plant specimens.

I thank my family and friends for their encouragement, deep concern and contributions in various ways to the success of my study.

I also extend my thanks and appreciations to the Biology department and Postgraduate and Research Program of Addis Ababa University for letting me to attend the study and covering the costs of the thesis research.

Last but not least, I acknowledge and thank the Almighty God; who gave me the opportunity to complete this study.

ABSTRACT

The karyotype of five endemic *Kniphofia* species of Ethiopia were studied. The studied species are: *K. foliosa, K. schimperi, K. hildebrandtii, K. isoetifolia, and K. insignis*. Somatic chromosomes were prepared from the root tip meristem using colchicines, 8-hydroxyquinoline or ice-cold pretreatment, followed by fixation (3:1, ethanol:acetic acid) and then enzyme maceration in pectinase-cellulase solution at 37°C for about 1 hr. Air-dry slides were prepared and stained in Giemsa stain in Sorensons phosphate buffer (pH = 6.8). The karyotype analysis has shown that *K. foliosa, K. schimperi, K. hildebrandtii and K. isoetifolia* are diploid with 2n = 12, where as *K. insignis* has 2n = 22 chromosomes which is assumed to be a polyploid on the basis of x = 6 for the genus. Polyploidy is the first report for the species. Morphologically, the chromosomes showed a gradual decrease in size in all the species with only small differences between the longest and the shortest chromosomes of each species. The size differences are relatively more pronounced in *K. insignis*. The karyotype formula was found to be 2m + 8sm+ 2st in *K. foliosa, K. schimperi and K. hildebrandtii*; 2m + 2m/sm + 8sm for *K. isoetifolia* and 4m + 12sm + 1sm/st + 5st for *K. insignis*. Generally, there is only a minor degree of differentiation observed among the karyotypes of the diploid species. One pair of small satellites were observed on the tip of short arms in *K. foliosa* and *K. hildebrandtii*, two pairs in *K. schimperii* and *K. isoetifolia* and in *K. insignis,* the secondary constriction is located in the middle of the long arm of a pair of chromosomes and thus delineated two large satellites. *Kniphofia foliosa* from 'Laga Shore', about 10 kms from Gedo town, west of Addis Ababa, was found to have a B-chromosome. This is the first report of B-chromosome for the species as well as the genus. In spite of the fact that samples were collected from only a limited number of sites, the cytogenetical information reported here would create only the basis for further cytological investigation of *Kniphofia* species. It is, thus, recommended that further cytological and molecular studies including specimens from a wider geographic area should be carried out.

Key words: B-chromosome, Chromosome, Endemic *Kniphofia* species, Ethiopia, Karyotype, Polyploidy.

1. Introduction

The present study deals with five Ethiopian endemic species of the genus *Kniphofia* Moench collected from different parts of the country. The genus *Kniphofia* Moench (Red hot poker) is categorized under the family Asphodelaceae.

1.1 The family Asphodelaceae

Previously the family Asphodelaceae was placed under the order Aspargales (APG, 1998) and considered to be a family of the lower asparagoids, which are characterized by simultaneous microsporogenesis (Chase *et al.,* 1995). However, the angiosperm phylogeny group (APG, 2003) tried to simplify the Aspargales classification, and has proposed that Asphodelaceae be included in Xanthorrhoeaceae to facilitate and simplify the teaching of asparagoid families. The APG classification is in a state of continual refinement and future changes are anticipated judging from the uncertainties and complexity in the classification of Asparagales (Ramdhani *et al.,* 2006).

According to Dahlgren *et al*. (1985), Asphodelaceae includes seven genera and around 450 species and it is fundamentally an Old World group with most of the genera occurring in sub-Saharan Africa. Smith and van Wyk (1998) reported that the family Asphodelaceae includes two sub-families: Asphodeloideae and Alooideae. These two sub families contain 17 genera and about 780 species that are mostly herbaceous and woody with trunks reaching to several meters high. They are mainly distributed in arid and mesic regions of the temperate, sub-tropical and tropical zones of the Old World in which South Africa is considered to be the main center of distribution.

Cronquist (1981) included *Kniphofia* Moench along with alooid genera under Asphodelaceae but later Dahalgren *et al.* (1985) classified the *Alooid* genera in the sub-family Alooideae and *Kniphofia* under Asphodelideae.

The identification of Knipholone type compound, anthraquinones, from the rhizomes of 46 species belonging to the subfamily Asphodelideae in the genera *Asphodelus, Asphodeline, Bulbine, Bulbinella*, and *Kniphofia* enabled scientists to separate the genera *Bulbine, Bulbinella and Kniphofia* from the rest of the genera of the sub-family and to conclude that the genus *Kniphofia* is not related to the *Alooideae* (van Wyk *et al*., 1995). In addition, van Staden and Drewes (1994) and Ermias Dagne and Steglich (1984) isolated anthraquinone knipholone from fresh bulbs of *Bulbine latifolia* and *Bulbine frutescens* that supports the placement of *Kniphofia* and *Bulbine* within the sub-family Asphodeloideae.

The presence or absence of thin-walled paranchymatous cells in the inner bundle sheath was also used to show the difference between the genus *Kniphofia* and the other genera of the sub-families and it was found that, all species under the genera have a thin-walled secretary tissue which suggests the relationship among all, except the genus *Kniphofia,* in which the inner bundle sheath is a lignified sclerenchymatous cells. This makes it different from the other group (Beaument *et al*., 1985).

The presence of tubular flowers and fused perianth was also used as another reason for the separation of *Kniphofia* from *Alooideae* (Smith and van Wyk, 1992).

1.2. The genus *Kniphofia* Moench

The genus *Kniphofia* was named in honer of Johannes Hieronymus Kniphof (1704-1763) who was a professor of medicine at Erfurt University in Germany (http://www.plantzafric a.com/plantklm/**kniphofias**.htm).

The genus *Kniphofia* Moench, commonly known as ‘red hot pokers’, belongs to the family Asphodelaceae in the sub-family Asphodeloideae (Smith and van Wyk, 1998). Berger (1908) monographed *Kniphofia*, recognizing 67 species with 13 varieties. Later, Janaki-Ammal (1950) indicated 93 species in which almost all were located in Africa, except a few species in Yemen. Codd (1968) indicated that the genus *Kniphofia* is represented by 70 species, of which 45 species occur in South Africa, 1 species in the Arab Republic of Yemen, 2 in Malagasy

Republic and 23 in Tropical Africa, including seven species in Ethiopia. But, later on, Ramadhani (2007) recognized that, the genus comprises around 71 species by including *K. monticola* which was not previously included by Codd (1968) and Kativu (1996). In addition, Ramadhani *et al.* (2006) pointed out that the species are almost entirely African, with two species from Madagascar and one species from Yemen, and they are chiefly distributed in southern and eastern Africa, preferring temperate mountainous grass-lands and moist habitats.

1.2.1. **Botany and distribution of the genus *Kniphofia***

The genus *Kniphofia* is generally perennial, acaulescent and herbaceous, caespitose or solitary, has a simple or branched thick rhizome and rarely a thick well- developed stem. The leaves are in a basal rosette, usually in 4 or 5 ranks, and rarely 2- ranked, linear, tapering gradually to the apex, usually keeled. The leaf margin is smooth to minutely serrulate. The genus also has an inflorescence peduncles that are terminal, stout, erect, sub-equal to the leaves, simple or very rarely branched, necked except for occasional sterile bracts below the inflorescences and inflorescence that are sub-capitate racemes of usually numerous flowers, dense or somewhat lax. Bracts are scarious or brown, persistent, longer than the pedicels. The pedicels are short to almost absent and articulated at the apex and flowers are spreading or pendulous with white, yellow or various shades of red. The perianth is tubular, campanulate to cylindrical or somewhat funnel-shaped and short, sub-equally lobed. The stamens are usually as long as or longer than the perianth at anthesis and the ovary is sessile, ovoid, 3-locular with many ovules in each locule. The fruits are globose to ovoid often 3-angled with loculicidal dehiscence and seeds are somewhat flattened, acutely 3-angled or winged (Sebsebe Demissew and Nordal, 1997).

In *Kniphofia*, the underground part consists of a thick rhizome and fibrous, fleshy roots. In some species the rhizome divides forming groups of stems, while in others the stems are more or less solitary (Eliovson, 1984).

Ramadhani *et al*. (2006) established that the genus *Kniphofia* has six centres of diversity, five of which are centres of endemism. The South African Centre is the most important in

comprising many species and is also the largest centre of endemism. According to Marais (1973), the genus *Kniphofia* shows variation in size according to the site and availability of water and occupies habitats that range from low and wet savannah grassland (from about 900 m a.s.l.) to montane and alpine vegetation (about 4,400 m a.s.l.). *Kniphofia* shows a strong afromontane grassland affinity in tropical and East Africa. In South Africa, it is found from high altitudes to coastal habitats, with the most species regions being afromontane grasslands. It is, thus, not considered to be an afromontane element, but rather an afromontane associate.

Five major evolutionary lineages were identified using cpDNA sequence data (*trnT-L* spacer), four of which are southern African. The fifth lineage was represented by material from Madagascar, tropical and East Africa (Ramadhani *et al*., 2006).

1.2.2. **Reproduction**

Kniphofia can be propagated sexually by seed. However, the low number of sexually reproducing plants in all species may affect the number of seedlings obtained from seeds which may have a negative consequence on the long-term survival of the plants. In addition, all *Kniphofia* species are obligate outcrossing, and the decline in new seedlings could result in the decline in the production of seed because of gametophytic self-incompatibility. It can also reproduce asexually by the division of underground stems called short rhizomes which can give to ramets, a potential to be physiologically independent. As a result, the asexual mode called vegetative reproduction has a more significant contribution to the growth of the population size even though it produces all genets in an area with the same genetic constitution due to proliferation and later fragmentation into clones (Tilahun Teklehaymanot, 2001).

1.2.3. **Economic Importance**

The genus *Kniphofia* is useful in the field of horticulture and it is grown in home and botanical gardens. Naturally occurring species of *Kniphofia* are important as honeybee plants for pollen source and nectar (Fichtl and Admassu Adi, 1994).

An infusion of the roots is used to relieve or treat chest disorder and *K. parviflora* is reported to have been made into a traditional snake repellant (Eliovsen, 1984). The roots of *K. foliosa* are used in traditional Ethiopian medicine for the treatment of abdominal cramps (Abraham Abebe *et al.*, 2005). In addition, knipholone and related natural phenyl anthraquinones extracted from the roots of *K. foliosa* are among a new group of potential anti-malarials and anthraquinone aloe-emodin was known to exhibit anti-leukemic properties (Ermias Dagne and Steglich, 1984; Esayas Berhanu *et al.,* 1986; Bringmann *et al*., 1999). Abraham Abebe *et al*. (2005) identified five compounds from the roots of *K. foliosa* among which 10-hydroxy-chrysopanol-9-anthrone and chryslandicin (anthraquinone–anthrone dimmers) were found to show a high inhibition of the growth of the malaria parasite, *Plasmodium falciparum* with a very little host cell toxicity.

1.2.4. **Conservation**

Many *Kniphofia* species are in urgent need of conservation because a high number of South African species are included in the red data list of Hilton–Taylor (1996). Scott-Shaw (1999) documented 17 *Kniphofia* taxa which are considered to be under threat in Kwazulu Natal (South Africa) and neighboring regions (Remdhani, 2007). The endemic *K. hildebrandtii* in our country, Ethiopia, also needs a special attention because of its distribution which is ecologically very restricted and located in highly venerable grassland which is used as a grazing land for cattle. In addition, *K. insignis* which is common on water logged habitats also needs a special attention because the community is changing the water logged sites into farmlands so that it will not have refuge places to escape (Tilahun Teklehaymanot, 2001).

1.3. The genus *Kniphofia* Moench in Ethiopia

Sebesebe Demeissew and Nordal (1997) recognized seven species of the genus *Kniphofia* in the Ethiopian flora. These are *K. foliosa* Hochst., *K. hildebrandtii* Cufod., *K. insigns* Rendle., *K. isoetifolia* Hochst., *K. pumila* (Ait) Kunth., *K. schimperi* Baker. and *K. thomsonii* Baker. Of these, *K. foliosa*, *K. hildebrandtii*, *K. insignis*, *K. isoetifolia* and *K. schimperi* are endemic to Ethiopia whereas *K. pumila* and *K. thomsoni* are widely distributed from West Africa to

eastern and central Africa. According to Marias (1973), *Kniphofia thomsoni* is common in Kenya, Uganda, and Tanzania, in particular on Mount Kilimanjaro.

Distribution wise, the endemic species are located between 6° 00' N to 14° 00' N latitude and 33° 00' E to 41° 46' E longitude (from 2000-4000 m a.s.l.) that falls within the mountainous areas of Ethiopia with disjunct distribution. Their habitat varies from montane grassland to sub-alpine *Erica arborea* zone (Tilahun Teklehaymanot *et al.*, 2004).

1.3.1. ***Kniphofia foliosa* Hochst.**

Kniphofia foliosa is a robust clump-forming herb with thick, erect, yellow rhizomes, and above ground stems reaching up to 40 cm, and with some fibrous remains of leaves at the base. Leaves are long-linear or lanceolate wide, keeled but apparently not deeply channeled, dark green to grey green in colour, margins serrulate, keel smooth below, serrulate near the tip. Raceme 15-40cm long, dense and cylindrical. Flowers are yellow, orange or red. (Marias, 1973; Sebsebe Demissew and Nordal, 1997).

This species best grows on well drained soil along road sides, overgrazed areas with scattered trees, hill sides, with rocky outcrops and tops of mountains ranging from 2500-4000 m a.s.l. (Sebsebe Demissew and Nordal, 1997). According to Maris (1973), the species is distributed in the mountain regions of central and northern Ethiopia and produces flower from June to September and also during December and January depending up on the availability of water. The plants cover larger areas at their place of occurrence (Tilahun Tekelehaymanot, 2001).

1.3.2. ***Kniphofia hildebrandtii* Cufod.**

Kniphofia hildebrandtii is a slender plant having only a few shoots, with fibrous remains of leaves at the base. Leaves are broad at the base, long-linear, dark, slightly greyish green, keeled and with smooth margins. Flowers are pale green or pale slightly greenish-white or yellowish, pendulous and usually found at one side. Bracts are ovate, acuminate or acute even in bud apparently dark, somewhat toothed or serrulate (Sebsebe Demissew and Nordal, 1997).

This distinct species is distributed in a relatively small area, ASgori, Ginch and Gedo, west of Addis Ababa, in Shewa along the road to Nekemet, growing in wet grassland at an attitude of 2000-2450 m a.s.l. and flowers from June to August (Sebsebe Demissew and Nordal, 1997; Marias, 1973).

1.3.3. ***Kniphofia insignis*** **Rendle.**

Kniphofia insignis is apparently solitary, slender, without fibrous remains of leaves at the base, with roots fusiform. Leaves are wide at the base, linear, long tapering, blunt at the tip with minutely keeled margin. Peduncle 20-65 cm long, sometimes reaching up to 100 cm. Racemes fairly dense in flower. Flowers are white or largely pale pink in color. Bracts are long, ovate-lanceolate. The species grows in flat areas, water-logged or flooded for part of the year during rainy period (Sebsebe Demissew and Nordal, 1997).

Kniphofia insignis is distributed in Northern Shewa Zone of Oromiya Region along the road to Debre Markos near Chancho, Rogore river, Ali Doro in Fitche at altitudinal range of 2550–3134 m a.s.l. (Tilahun Teklehymanot, 2001).

1.3.4. *Kniphofia isoetifolia* Hockst.

Kniphofia isoetifolia is generally apparently solitary, slender, sometimes in groups of five or six stems with little fine fibrous remains of leaves at the base. Roots are fusiform and leaves are wide, linear, blue–green, long tapering but the points themselves are blunt, soft and flaccid. Racems are dense or sub-dense in flower, capitate-globose to more or less long and centrifugal. Flowers are creamy, white, yellow, pale salmon, orange or red (Sebsebe Demissew and Nordal, 1997).

The plants produces flower from May to December (Marias, 1973). Flowers open from the top to the bottom while in the rest of the endemic *Kniphofia* species the flower opens from the bottom to the top (Marias, 1973).

Kniphofia isoetifolia is distributed in East Shewa Zone of Oromiya Region around Shashamane town, North Shewa Zone of Amhara Region, near Debre Birhan in 'Waf Washa' natural forest (Tilahun Teklehymanot, 2001), and western part of Arsi Zone of Oromiya Region, near Bekoji along Laga Kachama at an altitudinal range of 2050–3700 m a.s.l. The habitat of the species varies from water logged meadow in the montane highlands to sloppy ground of *Erica arborea* (Tilahun Teklehymanot, 2001).

1.3.5. ***Kniphofia schimperi*** **Baker.**

Kniphofia schimperi plants are slender, with fibrous remains of leaves at the base, probably forming small clumps with a compact, slightly branched root stock. Leaves are broad, linear, dark-green, long-acuminate, soft and reflexed and with almost smooth margins. Peduncle 43-130cm long and raceme secund. Flowers are yellow, orange, or red with bracts lanceolate, acuminate, scarious, white (Sebsebe Demissew and Nordal, 1997).

Kniphofia schimperi is distributed in the highlands of Wollo, at Abe Afetch, Bale at Dinsho, Gullale Botanic Garden (Entot Mountain) at attitudinal range of 2200-3500 m a.s.l. (Tilahun Teklehaymanot, 2001). The plants best grow on steep grassy or stony slopes, in rocky-outcrops, and stony banks near streams (Sebsebe Demissew and Nordal, 1997). The flowering period is from June to January (Marias, 1973).

1.4. Cytogenetics and its significance in plant systematics

Cytologic studies serve as sources of data for biologists concerned with systematics and evolutionary investigations (Cremer and Cremer, 1998). Cytological approaches to the systematics is largely based on chromosomes and cytogenetic analysis is almost always based up on morphological features of somatic metaphase chromosomes such as shape, length, centromeric position, telomers, satellites and nucleolar organizer regions (Singh, 2003).

Chromosomes are important in taxonomy because they have defined number that can be counted during mitotic and meiotic divisions; they have defined shapes that can be expressed in absolute and relative length units; they show landmarks such as centromere, nucleolar organizer regions, and heterochromatic bands which serve for individual chromosome identification; and their DNA content can be measured (Greilhuber and Ehrendorfer, 1988).

People have been using chromosome data for different purposes (Speicher and Carter, 2005). But, one of the most common reasons for chromosome study is to determine the identity of organisms or to sort organisms into different categories (De Melo *et al.*, 2000). Thus, chromosomes have been used to assign organisms to different taxa as members of the same species have similarity in their chromosome sets (Stace, 2000) whereas chromosome sets of different species may differ to variable degrees. Stace (1991) stated that, information from chromosome study is also useful in predicting reproductive property and/or fertility of organisms particularly in inter-specific hybrids. The chromosome complements of organisms can better be comprehended and useful inferences can be drawn when karyotype analysis is carried out.

1.4.1 Karyotype

Karyotype can be defined as the phenotypic appearance of the somatic chromosome complement of an organism. A karyotype can be constructed in various ways. The most often used approach is to cut out photographic images of somatic metaphase chromosomes from enlarged photographic prints, group them into homologous pairs and arrange the pairs in decreasing order of size (Greilhuber and Ehrendorfer, 1988; Stace, 2000). When carefully analysed, a given karyotype shows a number of features, which when taken together make the karyotype of one species distinct from that of other species. These features of karyotype include total and basic chromosome number, absolute and relative sizes of chromosomes, chromosome morphology, total chromosome length (genome size), and distribution of constitutive heterochromatic blocks that can be manifested upon C-banding, etc. (Levin 2002; Sharma, 1954). These attributes of karyotype are explained below under numerical and morphological features.

1.4.1.1 Chromosome number

The analysis of chromosome numbers represents an important step in the studies of genetic variation, phylogeny, taxonomy and evolution, as well as in the studies on the structure and diversity of genomes. Each species has specific chromosome number. The gametic chromosome number is expressed as n and the somatic number as 2n. In plant, the smallest 2n chromosome number so far recorded is 2n = 4 in *Haplopappus gracilis* (in the Family Asteracea) (Gupta, 2004) whereas the largest number is 2n = 600 in *Voanioala geradii* (in the Family Asteraceae) (Bennet, 1998).

Chromosome number is a product of two variables, the base-number (x) and ploidy level. The base number comprises one complete set of chromosomes. Organisms having only one set are referred to as monoploids; with two sets as diploids, and with more than two sets as polyploids. Most species are diploids (2n = 2x) or polyploids (2n > 2x). Polyploidy is rare in animals (Mable, 2004) and very common in all the major groups of plants except the gymnosperm (Lewis, 1980). It was indicated that, of the total studied plant species, at least 50% of world's higher plants and about 80% of grasses consist of polyploid taxa (Greilhuber and Ehrendrofer, 1988)

The sets of chromosomes possessed by a polyploid organism may be the same or comprised of two or more different sets. Thus, polyploidy can be categorized into either autopolyploidy in which the same chromosome set is multiplied or allopolyplody in which chromosome sets originate from different cross-hybridized parental species. Allopolyploids originate mainly from crosses between closely related species yielding homologous pairs of chromosomes (Stebbins, 1971).

Polyploidy has been a very important factor in the evolution of many plant groups and has in the past apparently resulted in the formation of a number of new species and several new genera (Grant, 1981). Thus, it plays important roles in the evolution and diversification of plants. Differences in ploidy level that diagnose each of a pair or group of species have been utilized as standard taxonomic information for many decades (Soltis *et al*., 2003). In addition,

polyploidy has important roles in the stabilization of several hybrid genotypes, providing a medium by which daughter and parent populations become immediately isolated from each other genetically (de Wet, 1971) and for obtaining fertile hybrids between species and genera (Sinha and Sinha, 1976).

1.4.1.2 Chromosome size

The most important concept in chromosomes is the variation in size between the chromosomes of a genome. The chromosomes in a genome can vary from being virtually all identical in size to exhibiting a size difference ratio of five or more (Stace, 2000). Such chromosome variations within the genome provided a powerful means of characterizing genomes by means of their relative sizes and shapes, giving rise to the concept of the karyotype and hence aid the cyto-taxonomic decisions (Gupta, 2004). Relative sizes of chromosomes are expressed in various ways such as expressing the size of individual chromosomes relative to the size of the smallest chromosome in the complement or relative to the total chromosome length of the complement.

Absolute chromosome size is another feature of the karyotype. Chromosome is normally measured at mitotic metaphase. Two species may have the same chromosome number, but they may differ in the absolute sizes of their chromosomes. The absolute size of chromosomes (chromosome length measured at metaphase stage) may be as short as 0.25 μm in fungi and birds, or as long as 30μm in some plants like *Trillium* with a variety of intermediate sizes (Gupta, 2004).

1.4.1.3 Chromosome morphology

Apart from variation in number and size, chromosomes vary greatly in their gross morphology, and this aspect has attracted much the attention of systematists. The most obvious features defining chromosome morphological variations are the following:-

1.4.1.3.1. Centromeric position

Centromer is the constricted region observed on the somatic metaphase chromosomes. The centromere is sometimes known as primary constriction. Functionally, a centromere serves as a point of attachment of spindle microtubules during cell division. It is considered as a very important morphological landmark for chromosome identification and characterization because it occupies constant position on a given chromosome but its position on different chromosomes may differ.

On the basis of its centromic position, a chromosome may be designated as metacentric, sub-metacentric, acrocentric and telocentric. As such categorization is often subjective and so lacks precision. Levan *et al.* (1964) suggested to define the position of the centromere based on the ratio of the long arm to the short arm of the chromosome (r = long arm/short arm). Thus, chromosomes are designated as M (median), m (median region), sm (sub-median), st (sub-terminal), t (terminal region), and T (terminal) with the corresponding arm ratios of 1.0, 1.0 - 1.70, 1.70 - 3.0, 3.0 - 7.0, 7.0 - ∞, and ∞, respectively.

1.4.1.3.2. Secondary constriction and satellites

Other morphological features of chromosomes include secondary constrictions and satellites. Like the centromere, secondary constrictions are under condensed regions of chromosomes, but unlike the centromere, depending upon the species, they occur on one pair or a few pairs of chromosomes only. These are the sites of ribosomal RNA genes (rDNA) and so the site where nucleoli are organized. Thus, they are sometimes referred to as nucleolar organising regions (NORs). Their position is frequently subterminal. The distal part of the chromosome arm that is separated by the secondary constriction is known as a satellite and a chromosome bearing a satellite is known as satellited chromosome (Stace, 1980). The presence of satellites helps to distinguish chromosomes bearing them from other chromosomes. If more than one pair of chromosomes are bearing satellite, the pairs can be distinguished from one another by the size or position of their satellites.

1.4.1.4 Karyotype symmetry

On the basis of relative chromosome sizes and centromeric positions, karyotypes may be classified as symmetrical and asymmetrical (Stebbins, 1971; Levitzky, 1931). A karyotype is said to be symmetrical if all the chromosomes are more or less of equal size and have median centromeres. A karyotype is asymmetrical when chromosomes are less uniform in size and centromeric positions are less median. Symmetry and asymmetry are used to measure karyoevolutionary trends in a given taxa (Lavania and Srivastava, 1992).

1.4.2 Karyotype Evolution

Normally, members of a given species possess similar karyotype, whereas, generally, karyotypes of different species differ from one another to a lesser or larger extent. The difference is less between karyotypes of related species than unrelated species. Since karyotypes of related species are assumed to have originated from the karyotype of a common ancestoral species, the difference between the karyotypes of related species must have come about through the process of karyotype evolution. Karyotypic differentiation is one of the key features in speciation. It is one of the mechanisms of post-zygotic reproductive isolation between biological species and as a result it contributes to the origin of new species (White, 1970).

In some cases, intrageneric differentiation in karyotypes is very pronounced. For example, in the genus *Crepis* interspecific differences regarding 2n number, base number, chromosome size (genome size) and karyotype symmetry have been described (Stebbins, 1971; Levin, 2002) and in the genus *Guizotia* differences in chromosome size among species (Hiremath *et al*, 1992) and asymmetry (Kifle Dagne and Heneen 1992; Kifle Dagne 1995) have been documented.

Karyotypes are dynamic structures and are in a state of change in the course of time. The changes involve chromosome number, centromeric position (arm ratios), number, size and position of secondary constrictions and associated satellites; absolute and relative size of the

chromosomes; position, number, size, and distribution of differentially staining heterochromatic segments. These changes comprise chromosome structural and numerical changes (Jackson, 1971; Stebbins, 1971).

1.4.2.1 Changes in chromosome number

Chromosome numerical changes are classified as aneuploid and euploid changes. Aneuploidy is characterized by the presence of larger or smaller chromosome number than that of individuals with the original chromosome complement but the change involves less than the basic chromosome number of the species. Aneuploid changes can be brought about in several ways. One such mechanisms is Robertsonian translocation (Robertson, 1916), which reduces the chromosome number by fusing two independent chromosomes into a single chromosome. Conversely, fission splits a biarmed chromosome into two independent chromosomes and thereby increases the chromosome number (Holmquist and Dancis, 1980; Schubert *et al*., 1991). Sometimes a chromosome gets lost after translocating its important genes to other chromosomes, resulting in the reduction of chromosome number (Stebbins, 1971). In addition to changes in chromosome number, fusion, fission and traslocations can alter the chromosome morphology by changing the location of the centromere or the arm ratio.

Euploid chromosome numerical changes involve gain or loss of complete set(s) of chromosomes. In diploids, gain results in polyploidy. If all of the sets belong to same genome, it is autopolyploidy and if the sets belong to two or more different species, it is allopolyploidy (Stebbins, 1971).

1.4.2.2 Change in chromosome gross morphology

In addition to size and number, chromosomes undergo gross morphological changes. These morphological changes are largely considered as chromosome structural rearrangements which are either qualitative or quantitative (Stebbins, 1971).

Qualitative structural rearrangements modify the chromosomal gene order but do not eliminate/duplicate any chromosomal region. The two general classes of qualitative structural rearrangements are translocation and inversion (Stebbins, 1971).

Translocations occur by breaks and transfer of a chromosome segment to another chromosome reciprocally or non-reciprocally. The latter type of translocation changes the sizes and the symmetry of the chromosomes involved. In reciprocal translocation, the chromosome morphology would not change if the sizes of the exchanged chromosomal segments are equal. This results in only change of linkage group of genes borne on the translocated segments. On the other hand, if unequal reciprocal translocation occurs, size and symmetry of the involved chromosomes would change.

Inversions occur when a segment of a chromosome is excised and reintegrated in opposite orientation into the same chromosome position, resulting in a reversed gene order. Inversions are called, pericentric when the centromere is included in the inverted region and paracentric when the centromere is not included. Pericentric inversion may change the position of the centromere and thus the arm ratio or chromosome symmetry if the break points in the two arms are not equally far from the centromere. On the other hand, paracentric inversion would not affect the centromeric position but it may change the chromosome morphology if other landmarks such as secondary constriction or satellite are included within the inverted segment (Stebbins, 1971).

Quantitative structural rearrangements include duplications and deletions that increase or decrease the gene dosage of a chromosomal region. Duplications are either tandem which occurs when the duplicated segments are adjacent to one another or insertional which occur when the duplicated segments are on different parts of the same chromosome or even on another chromosome. This phenomenon brings about karyotype evolution by affecting the chromosome size and centromeric position.

Deletions are losses of chromosome segments which could be interstitial, consisting of two breaks within a chromosome, resulting in the loss of an internal region, or terminal deletions

consisting of a single break, resulting in the loss of one of the ends of a chromosome. Deletion may result in change in structure of chromosomes. Deletions can produce changes in relative chromosome size, chromosome symmetry and in some cases reduce the chromosome number (Sharma and Sharma, 1959).

Most of the numerical and structural mutations of chromosomes are harmful and get lost from the population as soon as they are formed. On the other hand, advantageous or neutral mutations may stay in the population in which they are originated and ultimately get fixed, eventually establishing a new chromosomal race. The chromosomal race may ultimately evolve into a new species having a karyotype differing from that of the parental species in one or more of the karyotypic attributes discussed earlier (Levin, 2002).

1.5. B-Chromosomes

The earliest record of B chromosomes in plants was by Anne-Lutz in 1916, who discovered them in *Oenothera,* and referred to them as "diminutive chromosomes." The term B chromosomes was first used by Randolph (1928), to identify extra chromosomes in maize as not being duplicates of any of the basic A chromosome set.

B-chromosomes are among eukaryotic genomes composing selfish genetic elements which do not obey the Mendelian laws of inheritance (Camacho and Parker, 1994). B-chromosomes are dispensable extra chromosomes which are found in only some individuals of a population, and which are not duplicates of any member of the basic A chromosome set in diploids or polyploids. The Bs are not only extra, but they are also different and fail to pair or to combine with any of the A chromosomes at meiosis (Jones and Houben 2003; Kifle Dagne, 1995). They are often morphologically distinct, usually smaller than the As, and they can show numerical variation within and between individuals. The detection of B-chromosome rests on cytological observations. Phenotypic effects are usually unfavorable, especially with high numbers, and inheritance is irregular and non-Mendelian (Jones and Houben, 2003).

1.5.1. Distribution of B chromosomes

A more recent investigation of 23,652 angiosperm species (which consist approximately 11% of total estimated 260,000 species) revealed 979 species as carriers of B chromosomes: 8% being represented by monocots and 3% by dicots (Levin *et al*., 2005).

Within the same species, the number of B chromosomes varies from individual to individual. In some species they vary even between the organs, for example, in *Sorghum stipoideum* they are absent in stems and leaves but present in variable number in microsporocytes and tapetal cells (Wu, 1992) and in *Poa alpina* the Bs are present in primary roots and absent in adventory ones (Muntzing and Nygren, 1955).

The distribution of Bs between sexes also differs from species to species. Mostly Bs occur in both sexes, however, in some species the occurrence of Bs are higher in one of the sexes. In some species the Bs are present either in males or females only (Schmid *et al*., 2002). In addition, the analysis of 226 flowering plant species showed that Bs are predominantly present in outbreeding than in inbreeding species (Burt and Trivers, 1998).

Several factors like ploidy, genome size, breeding system, and altitude were found to be associated with the distribution of Bs across species. For example, it was confirmed on wild maize cultivars growing at higher altitudes to exhibit a higher number of Bs (Lia *et al*., 2007) and in *Astyanax scabripinnis* fish, located at different altitudes along the same river, B chromosomes were found in high altitude populations and were absent in low altitude populations (Neo *et al.,* 2000).

1.5.2 Origin and evolution of B chromosomes

There is no definitive elucidation of origin of B chromosomes (Camacho *et al*., 2000). Many cytologists believe that B-chromosomes are originated from A chromosomes (Dhar *et al*., 2002). Interspecific hybridization between closely related species on the other hand may give rise to B-chromosomes (Battaglia, 1964; Camacho *et al.,* 2000). In some cases, there are

indications that B-chromosomes originated from the sex chromosome of the A chromosomes (Green, 1990).

1.5.3 The role of B chromosomes

The B-chromosomes are devoid of any genetically active loci, and yet they have effect on their hosts. The effects of B chromosomes are usually cumulative, depending upon their number and not their presence or absence i.e. different phenotypic effects were correlated with presence of high number of Bs (Green, 1990). At low number, the B chromosomes do not have any influence on phenotype. In general, Bs influence negatively the fitness and fertility of the plants (Gonzalez-Sanchez *et al*., 2004). In maize for example, leaf stripping was correlated with the presence of Bs (Staub, 1987).

Not only negative effects are associated with B chromosomes but they can act as diploidizing agents in allopolyploids (Jenkins, 1986). In rice, for example, Cheng *et al.* (2000) observed a slight positive effect of Bs on plant height, weight of grain, length of panicle, length and weight of grain and negative effect on number of tillers and width of grain. In some species, individuals with Bs show better survival rate under certain stress conditions (Plowman and Bougourd, 1994).

In hybrids, Bs prevent or suppress the homologous pairing of A chromosomes as observed in hybrids of different *Aegilops* species (Evans and Davies, 1985).

1.6. Karyotype studies in *Kniphofia* Moench.

A preliminary cytological examination was made by Moffett (1930) on several species of *Kniphofia* and it was found that the basic (haploid) chromosome number was six (6) and chromosome pairing and separation at meiosis was perfectly regular for most species. However, Moffett (1930) observed, in some of the preparations, pollen mother cells containing 12 or 24 chromosomes scattered at random throughout the cell, which suggested that certain species of *Kniphofia* are tetraploid.

Webber (1932) examined the karyology of *K. aloides* (i.e. *K. uvaria*) in detail and found that there were six pairs of chromosomes (2n= 12). de Wet (1960) noted the somatic chromosome number of 2n= 12 in 17 species of *Kniphofia* (viz. South African representatives, including *K. typhoides,* which was previously placed in *Notosceptrum*) and chromosome morphologies showed little difference. Nayak and Sen (1992) examined the karyology of *K. nelsonii* (i.e. *K. triangularis*) and *K. uvaria* clones and reported that most of the cells examined were diploid (2n = 12), and some cells were tetraploid.

Tilahun Teklehaymanot (2001) indicated that the endemic *Kniphofia* species of Ethiopia have a diploid chromosome number (2n) of 12 with a karyotype formula of 1m + 3sm + 2st. In addition he indicated that the evolutionary differentiation of the *Kniphofia* species in Ethiopia has taken place without involving either change in ploidy levels or karyotype differentiation since the image analysis of the somatic chromosomes of the five endemic and one wide spread *Kniphofia* species agreed with the studies of Webber (1932) and de Wet (1960) except slight differences on the definition of the centromeric positions. de Wet (1960) defined chromosome VI as submedian but Tilahun Teklehaymanot (2001) found it to be subterminal. Webber (1932) defined the centromer of chromosome III as subterminal but Tilahun Teklehaymanot (2001) indicated as submedian.

In addition, isozyme analysis of the five endemic and one wide spread *Kniphofia* species indicated that, they have displayed higher genetic similarity parameters than other endemic plants irrespective of morphological variation particularly in floral morphology and inflorescence architecture. The study also indicated that the Ethiopian endemic *Kniphofia* species share a fairly recent common ancestor, but have differentiated in floral and inflorescence character through rapid evolution (Tilahun Teklehaymanot *et al*., 2004).

2. Objectives of the Study

2.1. General Objective

- To characterize the five endemic *Kniphofia* species with regard to their chromosome cytology

2.2. Specific Objectives

- To determine the chromosome number of the five endemic *Kniphofia* species.
- To construct the karyotypes of the five species.
- To describe the karyotype of the five endemic *Kniphofia* species.

3. Materials and Methods

3.1. Plant materials

In the present study, five endemic *Kniphofia* species of Ethiopia, collected from their natural habitats in different parts of the country, were investigated with the main emphasis on somatic chromosomes.

Sample specimens of these species were transplanted into pots in the glass-house at the Science Faculty of Addis Ababa University. Fresh root tips were used for somatic chromosome study. The chromosome counts were based on maximum counts from more than one intact cell of actively dividing root tips. The *Kniphofia* species used in this study are listed in Table 1.

Table: 1. Species names and collection sites.

Species	Site of collection
K. foliosa	Gefersa-20 kms, West of AA, Laga Shore-10 kms, West of Gedo town, Bale Mountains National park-350 kms, South East of AA
K. schimperi	Intoto mountain-10 kms, North West of AA; Bale Mountains National park (H.Q)*-350 kms, South of AA.
K. hidebrandtii	Asgori-88kms, West of AA; Gedo (Gura Forest)-198 kms, West of AA.
K. isoetifolia	Bekoji (Laga Kechema)-50 kms, South East of Asella.
K. insignis	Chancho-40 kms; Fitche-116kms, North West of AA

Note:- *H.Q = Head Quarter

A

B

C

D

E

Figure 1 *Kniphofia* specimens : *K. foliosa* (A), *K. schimperi* (B), *K. hildebrandtii* (C), *K. insignis* (D), *K. isoetifolia* (E).

3.2. **Pretreatment of roots for chromosome preparation**

For somatic chromosome studies, actively growing root tips from the potted plants were harvested separately from individual specimens growing in the greenhouse. The root tips were either cold treated, by keeping them in ice water for 28 to 32 hours, or treated with 0.05%

colchicine, or (8-hydroxyquinoline, 0.002M) for 2 to 4 hours at room temperature. These treatments arrest the chromosomes by poisoning the spindle microtubules at metaphase stage and as a result the number of cells at this stage increases. The root tips were fixed in 3:1 (ethanol:acetic acid) for 24 hours. After rinsing in distilled water, enzyme maceration was performed in 4% cellulase plus 4% pectinase solution in a water bath for about 1 hour at 35-37°C. As a resuit, the lower meristematic tip of the root detaches from the root tip on little agitation (Kifle Dagne and Heneen, 1992). After decanting the enzyme solution, the root tips were rinsed in several changes of distilled water (Kifle Dagne, 1994) before using for air-dry slide preparation.

3.3. Air-dry slide preparation

The detached (enzyme macerated) meristematic tip of the root was pipetted on to a glass slide. The excess water was blotted off from around the root tips using a filter paper (by touching the edge of the water drop) and root tips were mashed in a drop of fresh fixative (3:1, alchohol:acetic acid) and the cells were spread by strongly blowing on the slide.

Permanent slide preparations were made by staining air dried preparation in Giemsa stain in Sorensen's phosphate buffer (pH = 6.8) for about 45 minutes to 1 hour, followed by rinsing in the same buffer, air drying and mounting in DPX.

The slides were then analysed under a light microscope for well spread metaphase chromosomes and photographs were taken. Karyotypes were made from enlarged photomicrographs of the somatic chromosomes. The nomenclatures used in defining the centromere position (M, m, sm, t, st, and T) were adopted from Levan *et al*. (1964). Chromosome size and arm ratio measurements were on enlarged photomicrographs.

4. Results

In the present study, the chromosomes were best counted and characterized at c-metaphase stage that usually allows accurate counts and observation of the chromosome morphology since, at this stage, the chromosomes become distinct and separated. The findings of the study are presented in the following sections:

Table 2 Summary of chromosome counts of the studied species

Species	Number of potted plants analyzed	Number of metaphase plates analyzed	2n chromosome number
K. foliosa	3	8	12+1B
K. schimperi	3	10	12
K. insignis	6	12	22
K. hildebrandtii	3	12	12
K. isoetifolia	4	8	12

4.1. *Kniphofia foliosa*

In the present study, *Kniphofia foliosa* was found to have a somatic chromosome number of 2n = 12, which was diploid. The chromosomes showed a gradual decrease in size from the longest to the shortest (Figure 2B) but with a narrow range of mean size of 14.4 µm to 20.0 µm (Table 4) for the longest and shortest pairs, respectively. Morphologically, chromosome pair I was m type, pairs II, III, V and VI were sm type and pair IV was st type.

In addition to the basic chromosomes or the A chromosomes, it was found that *K. foliosa* specimens collected from 'Laga Shore' West Shewa Zone, at about 10 kms from Gedo town along the road to Fincha Sugar Factory, possess a small supernumerary chromosome (B-chromosome) indicated by an arrow in Figure 2A. This chromosome had a size of about 2.941µm and a median centromere. The B-chromosome showed a normal disjunction as observed at anaphase stage of mitosis of the root tips (Figure 4).

Thus, the karyotypic formula of the species is 2 m + 8 sm + 2 st or 2 m + 8 sm + 2 st + 1B, where B chromosome is present. Small satellites have been observed at the termini of the short arm of a pair of chromosomes (Figure 3).

The species has a large genome with total chromosome length of about 106 µm per haploid genome (Table 4). The average asymmetry is about 2.068 (Table 5), which means that the average centromeric position is in the sub-median region (sm). On the average, the karyotype is asymmetrical when considered from the centromeric position point of view.

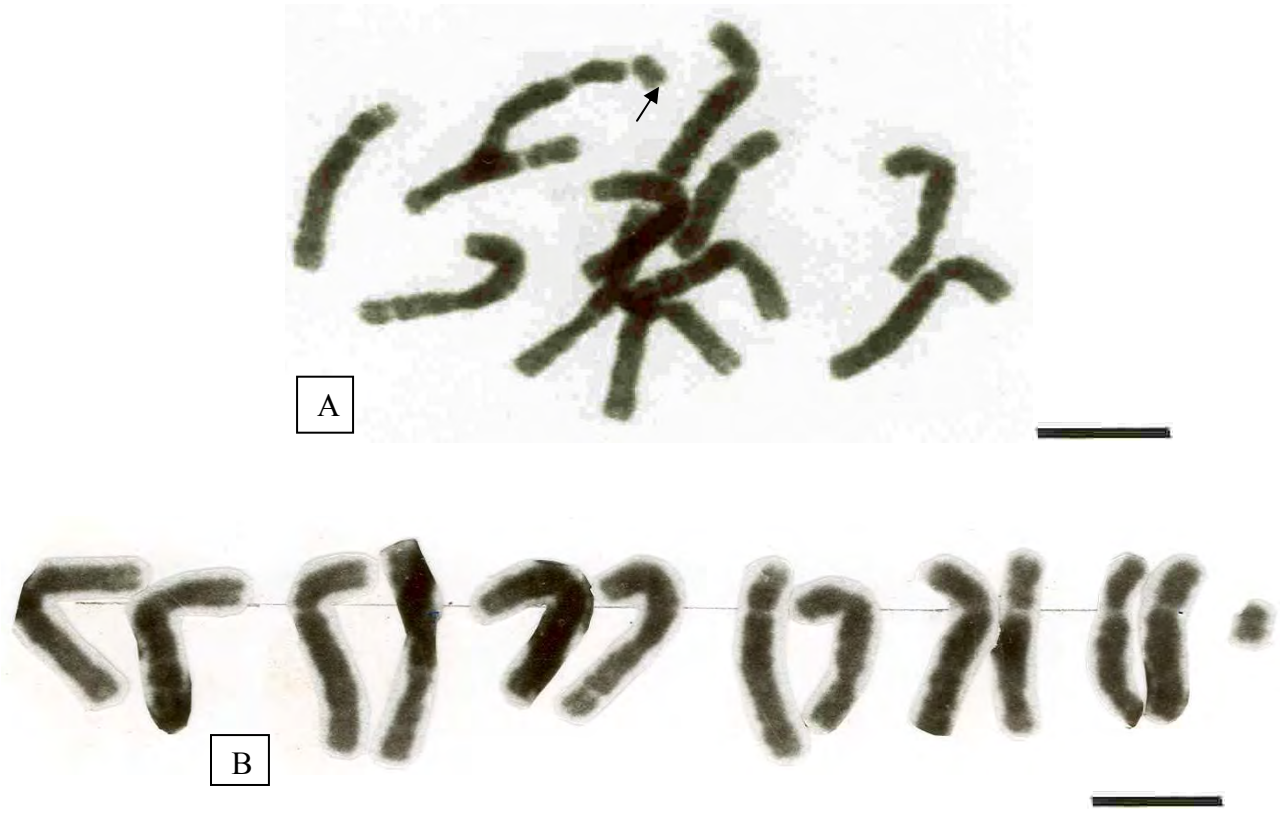


Figure 2 Metaphase chromosome spread (A), B-chromosome (arrow) and karyotype (B) of *K. foliosa.* Bar=10µm

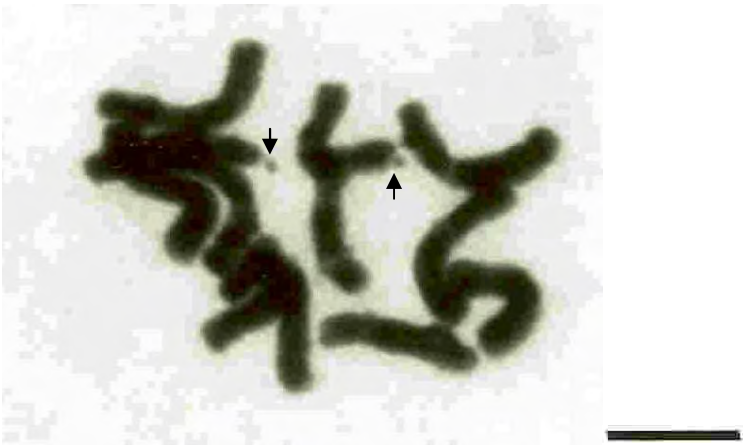


Figure 3 Chromosome satellites (arrow) of *K. foliosa*. Bar = 10µm

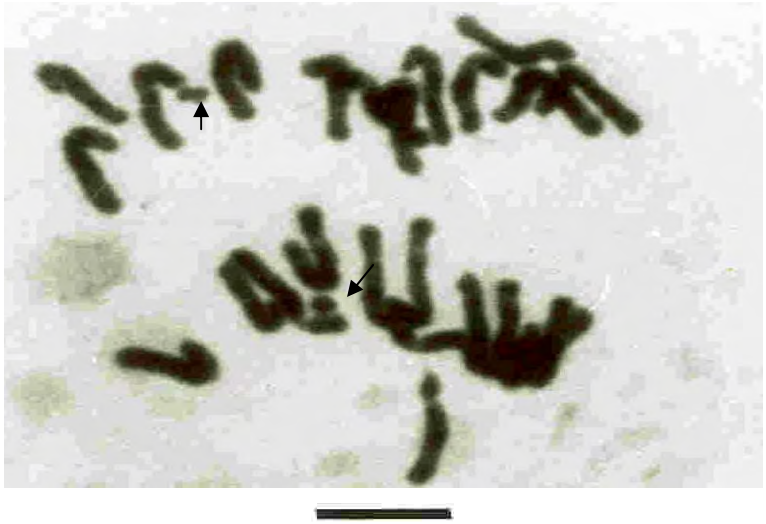


Figure 4 Mitotic anaphase stage showing normal disjunction of the B-chromosome (arrows) of *K. foliosa*. Bar = 10µm.

4.2. *Kniphofia schimperi*

In this study, the diploid somatic chromosome count for *K. schimperi* was found to be 2n = 12 (Figure 5A). The centromeric positions of the chromosomes are median region (m) for chromsosme I, sub-median region (sm) for pairs II, III, V, VI, and sub-terminal region (st) for pair IV (Figure 5B ; Table 3).

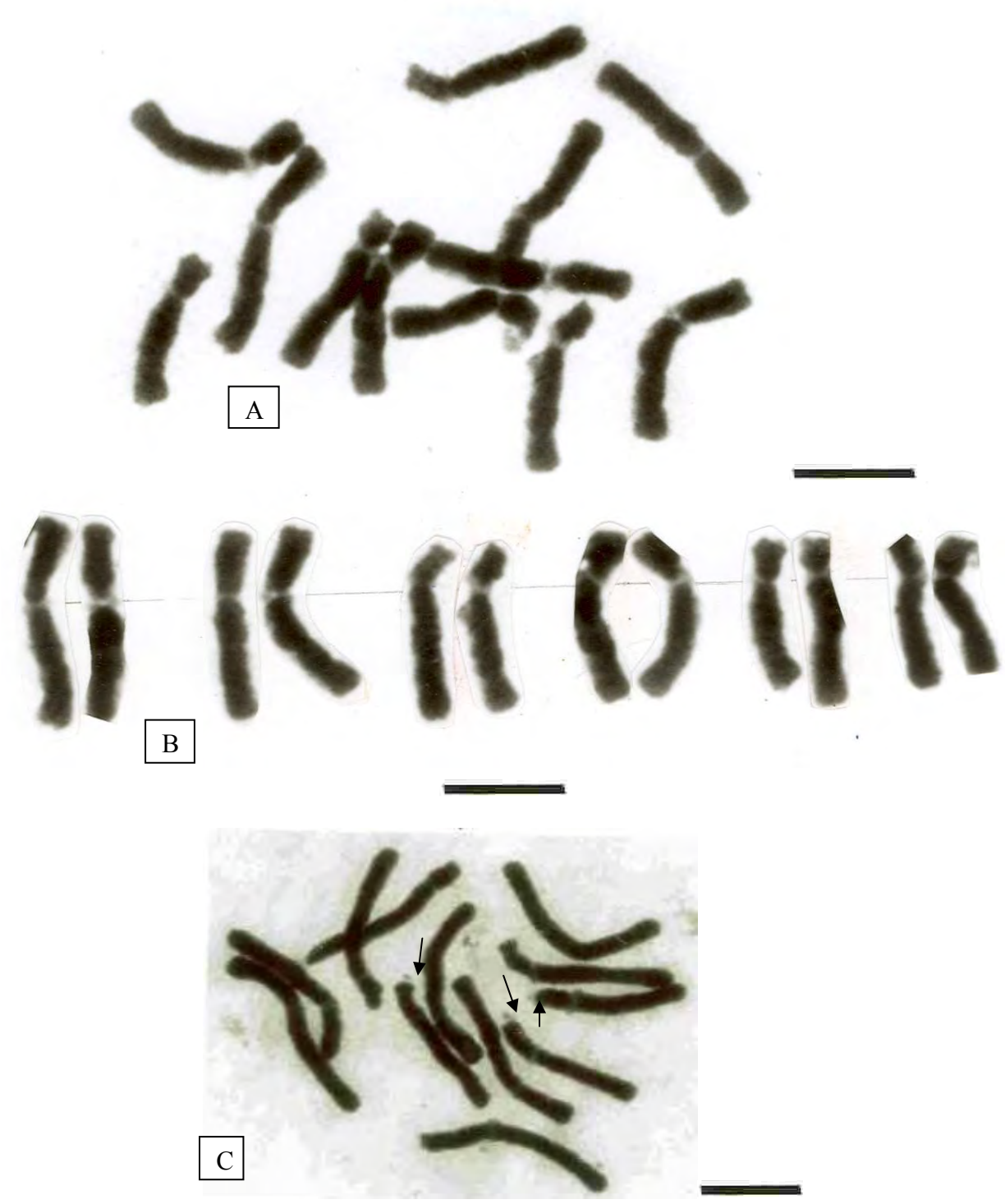


Figure 5 Metaphase chromosome spread (A), karyotype (B) and chromosome satellites (C) for *K. schimperi*. Bar = 10µm.

Thus, the karyotype formula of the species is 2 m + 8 sm + 2 st (Appendix 2). The mean chromosome length is 20.40 µm and 15.0 µm for the longest and shortest pairs, respectively (Table 4) with a total chromosome length of about 103.70 µm per haploid genome (Table 4). The average chromosome asymmetry, on the basis of centromeric position, is about 2.217, indicating that the average centromeric position is in the sub-median region (sm) (Table 5).

In *K. schimperi* three chromosomes were observed with satellite (Figure 5C) at the tip of the short arms which is indicative of the presence of two pairs of satellite chromosomes in this species.

Table 3 Mean arm ratios of the chromosomes and designation of centeomeric positions. Letters within brackets represent chromosome type.

	Mean arm ratio (L/S)										
Species	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
K. foliosa	1.47 (m)	1.98 (sm)	1.76 (sm)	3.11 (st)	2.41 (sm)	2.45 (sm)	-	-	-	-	-
K. schimperi	1.48 (m)	1.82 (sm)	2.72 (sm)	3.10 (st)	2.58 (sm)	2.52 (sm)	-	-	-	-	-
K. hildebrandtii	1.33 (m)	1.80 (sm)	2.14 (sm)	3.19 (st)	2.76 (sm)	2.63 (sm)	-	-	-	-	-
K. isoetifolia	1.44 (m)	1.67 (m)	2.27 (sm)	2.41 (sm)	2.47 (sm)	2.48 (sm)	-	-	-	-	-
K. insignis	1.28 (m)	2.37 (sm)	3.04 (st)	3.40 (st)	2.75 (sm)	3.33 (st)	3.00 (st/sm)	2.26 (sm)	2.18 (sm)	1.81 (sm)	1.39 (m)

4.3. *Kniphofia insignis*

In the present study, the chromosome count for *K. insignis* was found to be 2n = 4x = 22 (Figure 6A) which is assumed to be a tetraploids on the basis of x = 6 for the genus.

Morphologically, the size of the chromosomes decreases gradually but with a relatively wide range of size differences between the shortest and the longest pair of chromosomes in the karyotype, 10.7 µm to24.4 µm, respectively (Table 4). As shown in Figure 5B, the centromeric position of pairs I and XI are median region (m), pairs II, V, VII, IX, and X sub-median region (sm), and pairs III, IV, VI and VIII are sub-terminal (st). Thus, the karyotype formula of the species is 4 m + 10 sm + 8 st (Appendix 5).

In this species, it was observed that a pair of chromosomes has a distinct constriction at the middle of the long arms (Figure 7, arrows) which is probably a nucleolus organizer region. In less condensed chromosomes, the constriction is even very prominent (Figure 6B). Because of its position in the middle of the long arm, the constriction delimits off a large satellite with a size of about half the size of the long arm or one-third of the total chromosome length. There was no any other constriction or satellite observed on the chromosomes of this species.

Owing to its polyploid nature, this species has the largest genome of all the five species investigated in the present study, with a total chromosome length of about 164 µm. However, this was much less than double (only about three-fourth) the size of the total chromosome length of the diploid species. The average asymmetry of the chromosomes was about 2.266 (Table 5), which was somewhat more asymmetric than the average asymmetry observed for the other species of *Kniphofia* considered in the present study.

Table 4 Summary of the average chromosome length of individual pairs and total chromosome length per haploid genome of the studied species in µm.

	chromosome number and their mean size in µm from left to right											
Species	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	Total
K. foliosa	20.0	19.7	18.2	16.9	16	14.7	-	-	-	-	-	105.5
K. schimperi	20.4	18.7	16.9	16.3	16.3	15.0	-	-	-	-	-	103.7
K. hildebrandtii	18.8	18.5	17.1	16.6	16.0	14.3	-	-	-	-	-	101.3
K. isoetifolia	22.9	21.2	19.7	18.4	18.4	16.9	-	-	-	-	-	117.5
K. insignis	24.4	17.4	16.6	16.2	15.4	15.3	14.7	12.1	10.3	10.9	10.7	164.0

Note:- The Roman numerals represent chromosomes according to their decreasing size from left to right. The size for B-chromosome in *K. foliosa* = 3.24 µm.

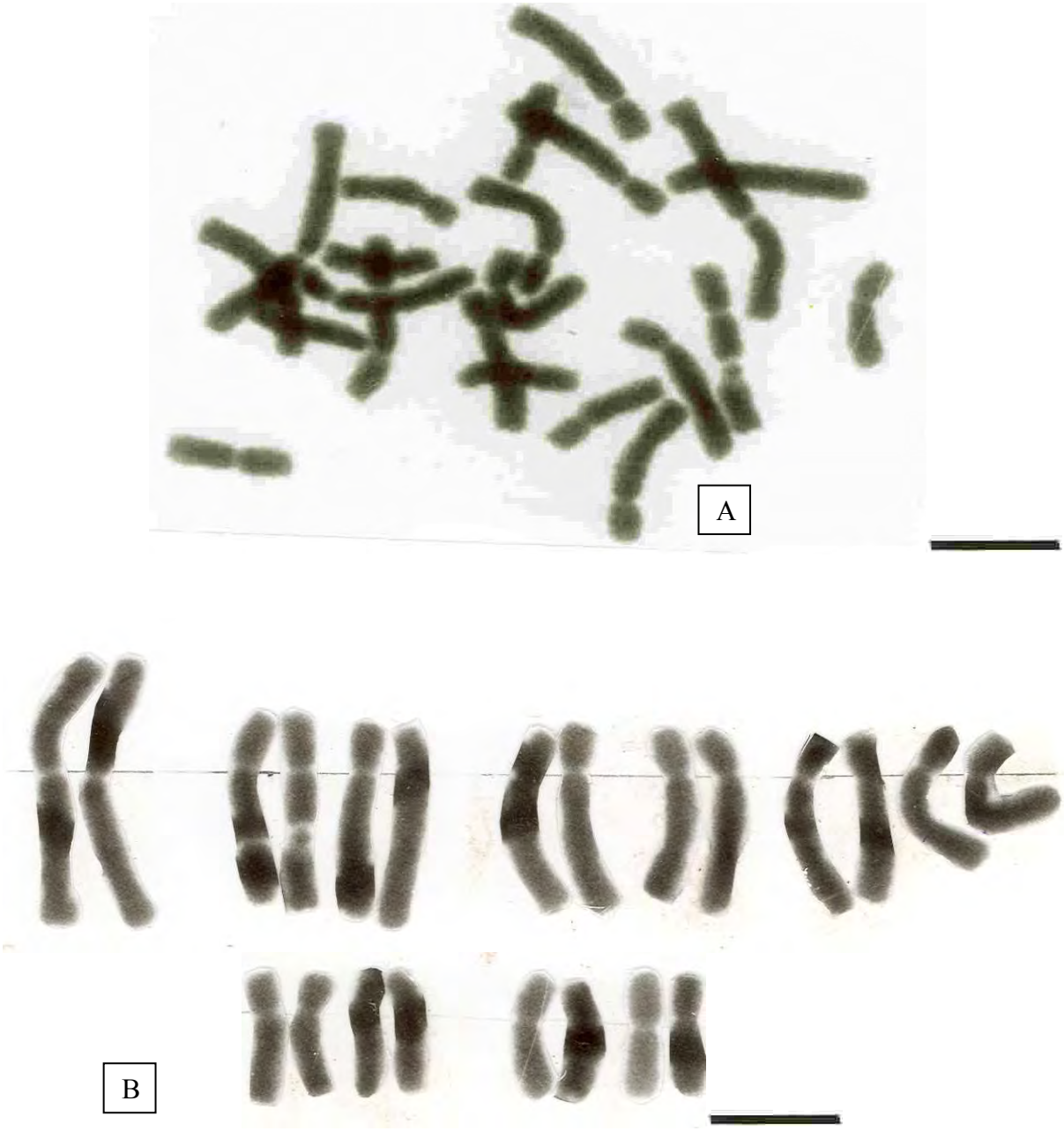


Figure 6 Metaphase chromosome spread (A) and karyotype (B) of *K. insignis*. Bar =10µm

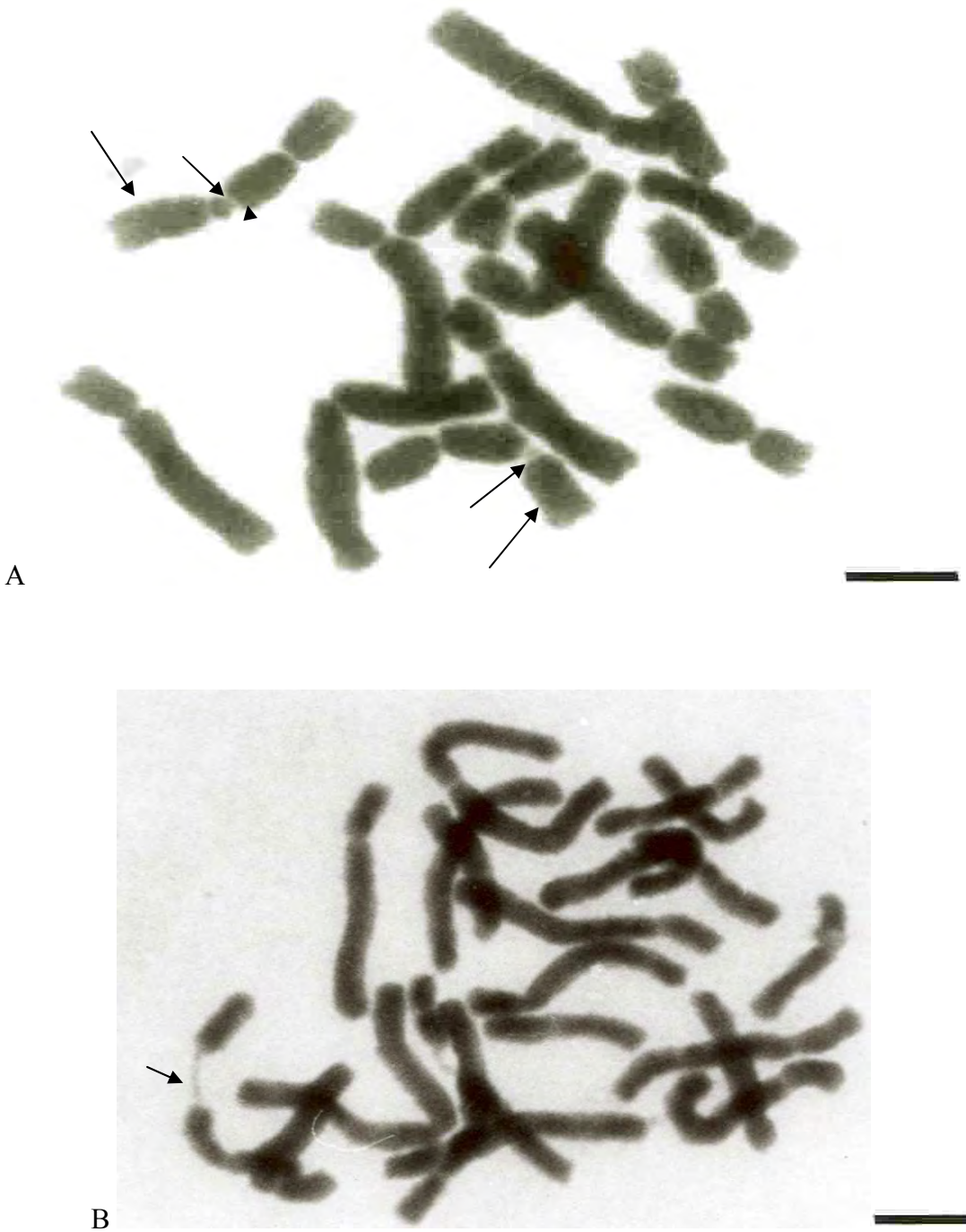


Figure 7 Chromosome of *K. insignis* showing secondary constriction. A. Partial complement with arrow head indicating secondary constriction, short arrows the satellite and long arrow showing region lying between two constrictions. B.Less condensed chromosomes showing a prominant secondary constriction (arrow). Bar = 10 µm.

4.4. *Kniphofia hildebrandtii*

The somatic chromosome count for this species was found to be 2n = 12, which is diploid (Figure 8A). The size of the chromosomes gradually decreases on karyotypying like as in the preceding species. The mean chromosome length ranges from 14.3 µm to 18.8 µm (Table 4).

The data showed that, the centromeric position of pair I is median region (m), pairs II, III, V, and VI is submedian (sm) and pair IV is subterminal (st). Thus, the karyotype formula of the species is 2 m + 8 sm + 2 st (Appendex 3).

In *K. hildebrandtii,* it was observed that a pair of chromosomes bears satellites at the tip of the short arms (Figure 9, arrows). The total chromosome length of this species is about 101 µm (Table 4), whereas the average asymmetry of the chromosome complement is 2.146 (Table 5).

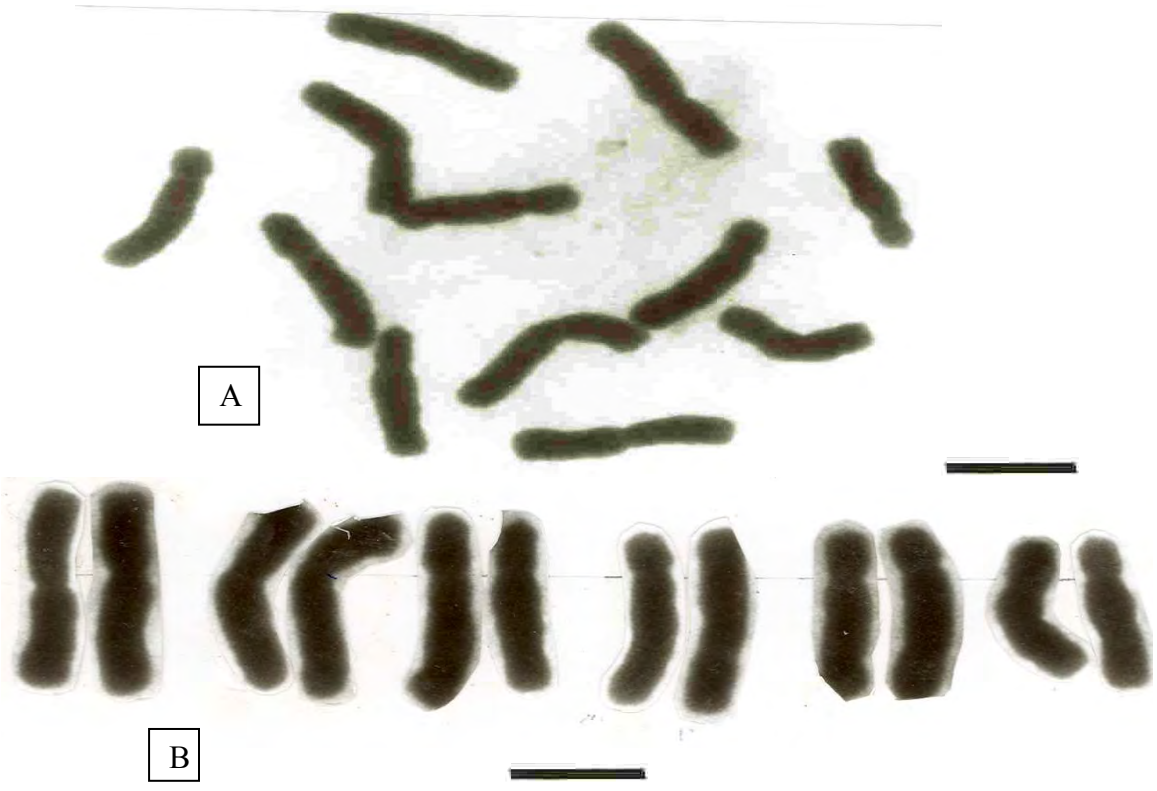


Figure 8 Metaphase chromosome spread (A), karyotype (B) of *K. hildebrandtii*. Bar = 10µm

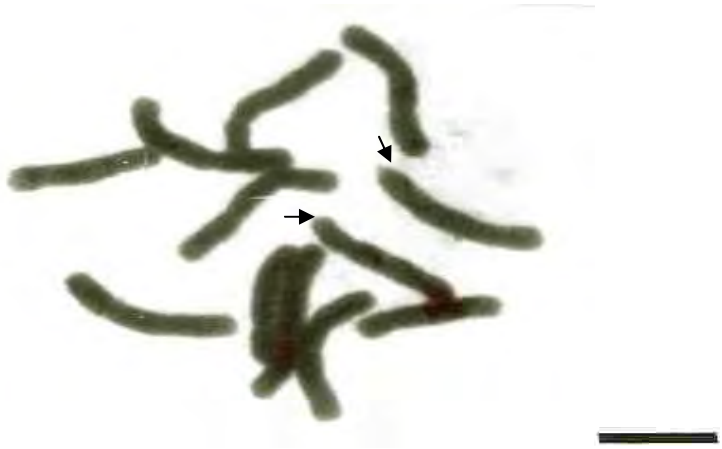


Figure 9 Chromosome satellites of *K. hildebrandtii*. Bar = 10µm

4.5. *K. isoetifolia*

The metaphase chromosome count in the present study for this species was found to be 2n = 12 (Figure 10A). The size of the chromosomes decreases gradually and the mean size ranged from 16.9 µm to 22.9 µm (Table 4). Regarding centromeric position, it was median region (m) for chromosome pairs I and II, and sub-median region (sm) for chromosome pairs III, IV, V and VI. Thus, the karyotype formula for the species was found to be 4 m + 8 sm (Appendix 4). The species was also found to bear satellites on the tip of the short arms of two pairs of chromosomes (Figure 11). *K. isoetifolia* had total chromosome length of about 118 µm (Table 4) which was slightly more than that of the diploid species investigated in the present study. The average asymmetry was about 2. 015 (Table 5).

Table 5 Average symmetry/asymmetry of chromosome complements of the five *Kniphofia* species based on the ratio of the total length of all the long arms to the total length of all the short arms of 2n chromosomes

Species	Total long arms	Total short arms	Total long arms / Total short arms
K. foliosa	24.2	11.7	2.068
K. schimperii	24.30	10.96	2.217
K. hildebrandtii	23.5	10.95	2.146
K. insignis	38.3	16.90	2.266
K. isoetifolia	26.70	13.25	2.015

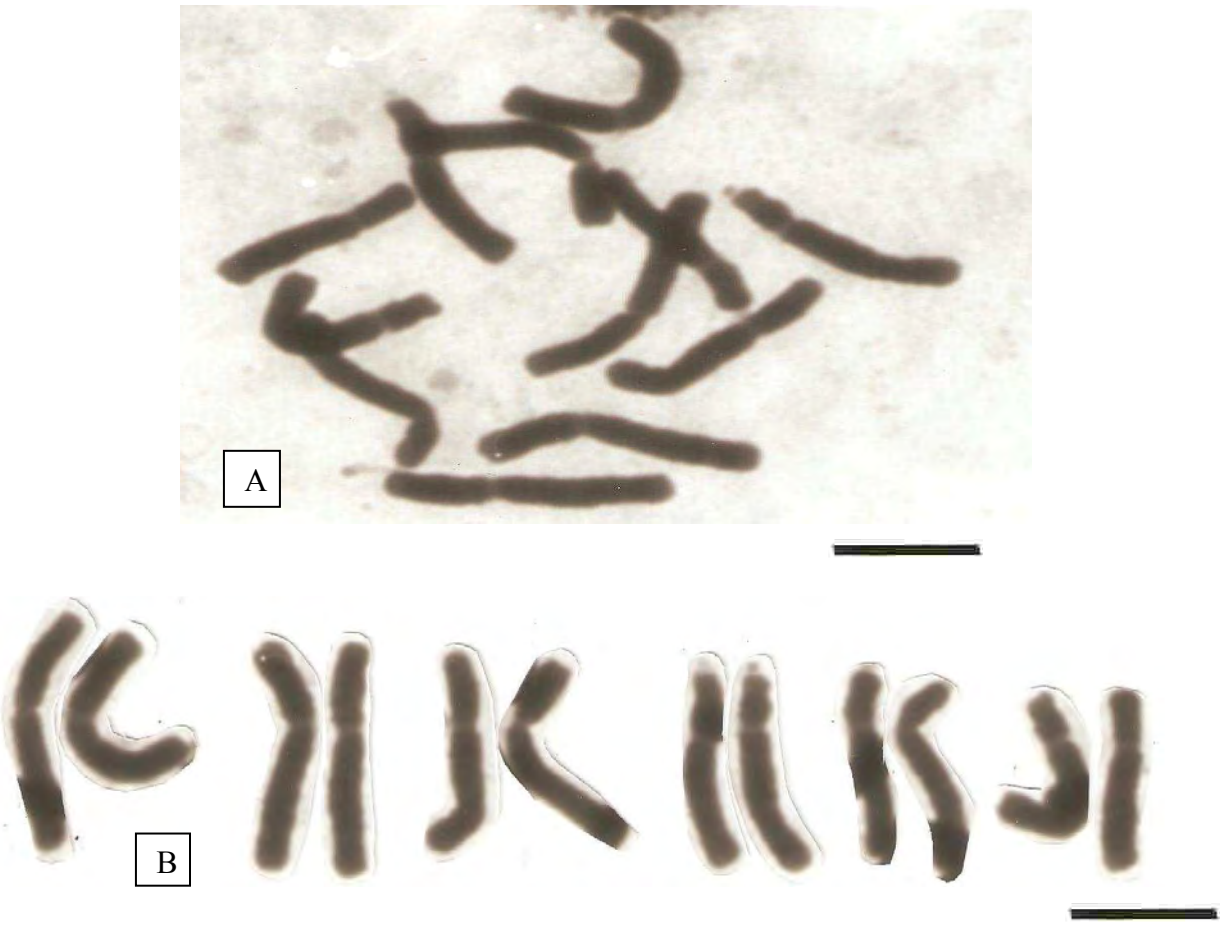


Figure 10 Metaphase chromosome spread (A) and karyotype (B) of *K. isoetifolia.* Bar = 10 µm.

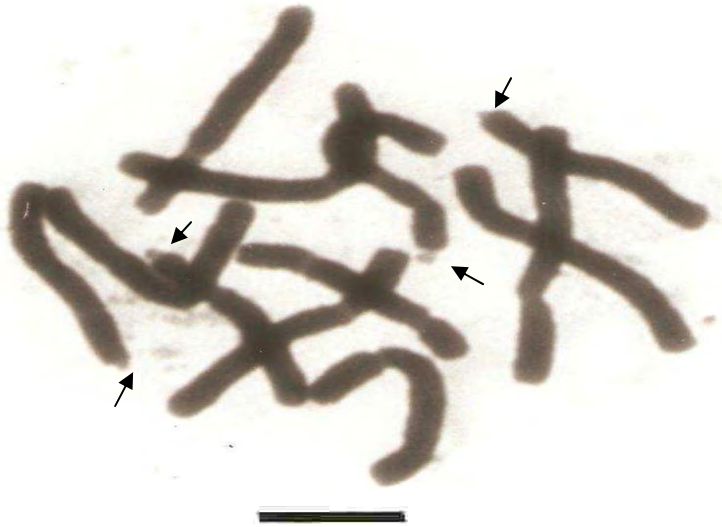


Figure 11 Chromosome spread of *K. isoetifolia* showing satellites (arrows)

5. Discussion

The Ethiopian endemic *Kniphofia* species, cytogenetically investigated in this study, were found to comprise of both diploids and polyploid species. Of the five endemic species investigated, four species (*K. foliosa, K. schimperi, K. hildebrandtii, and K. isoetifolia*) were found to be diploids with 2n = 12 and basic chromosome number of x = 6. This result is in agreement with the previous report by Tilahun Teklehaymanot (2001) on the same species and Webber (1932) and de-Wet (1960) on different species of the genus *Kniphofia* from South Africa. In the present study, specimen of *K. insignis* collected from Fitche 'Bullo Warke' and Chancho along Siblu river (North Shewa) were found to contain 2n = 22. This finding is at variance with the previous report by Tilahun Teklehaymanot (2001) in which all endemic species were reported to be diploid, 2n = 12. Moffett (1930) also reported polyploidy for a different species of *Kniphofia* from South Africa. This is the first report of polyploidy for the Ethiopian endemic *Kniphofia* species in general and *K. insignis* in particular.

Polyploidy, according to Otto and Whitton (2000), indicates rapid evolution of species and a particular polyploid individual eventually diverges from the rest of species having normal chromosome set because of reproductive isolation. At present, it is not clear whether the polyploid *K. insignis* is an autopolyploid or allopolyploid and this needs further testing. However, in the karyotype, the chromosomes are grouped into homologous pairs only, which implies that it is probably an allopolyploid. However, further studies such as meiotic chromosome pairing are necessary to determine the polyploidy nature of the species.

On the basis of x = 6 for the genus *Kniphofia*, one would expect the tetraploid species to have 2n = 4x = 24, instead of 2n = 22. The reduced chromosome number of *K. insignis,* can be explained by assuming an aneuploid reduction following the formation of a tetraploid with 2n = 24 chromosomes. Taxa with ascending and descending aneuploid series of chromosome numbers are often encountered in many polyploid lineages (Levin, 2002). Reduction in chromosome number can occur either through Robertsonian fusion followed by a loss of the small fusion product leaving the larger product behind. Another possible mechanism of reduction in chromosome number could be through translocation, i.e, when a chromosome

translocates most of its chromatin to another chromosome and then gets lost from the complement. A polyploidy with such a chromosome number can also arise without reduction in chromosome number provided that the diploid parental species have different basic numbers i.e. dibasic polyploidy. In the present case, if one parental species is 2n = 2x = 10 and the other is 2n = 2x = 12, the resulting allotetraploid will have 2n = 22. However, so far the basic chromosome number reported for the genus is x = 6. The assumption of dibasic polyploidy would lead to another assumption that there may be a *Kniphofia* species with x = 5.

In addition to having a different ploidy level, the satellited chromosome pair of *K. insignis* is morphologically distinct from that of the other species studied here. Unlike that of the other species, satellited chromosomes of *K. insignis* have secondary constriction in the middle of the long arm which delineates a large satellite, about one-third of the total length of the chromosome or half of the length of the long arm (Figure 7A) whereas the satellites in the other species are very tiny and are located at the termini of the short arms. One may make several speculations about the origin of such a satellite chromosomes. One possible origin of such chromosome could be paracentric inversion in which the secondary constriction is included in the inverted segment. This, however, does not reduced the chromosome numbers by itself from 2n = 24 to 2n = 22. Another possibility is a terminal translocation of a large chromosome segment on to the satellite end of a satellited chromosome. If, after the translocation, the donor chromosome is lost, this process would at the same time explain the reduction of the chromosome number from 2n = 24 to 2n = 22. A look at Figure 12 gives an impression that the satellite chromosome was formed through a translocation of a large chromosome segment on to a satellited chromosome. As can be seen from the figure, the satellite has a narrow region adjacent to the secondary constriction (arrow head) and a broader region (short arrow). One may speculate that the former is the original satellite and the later is a translocated segment.

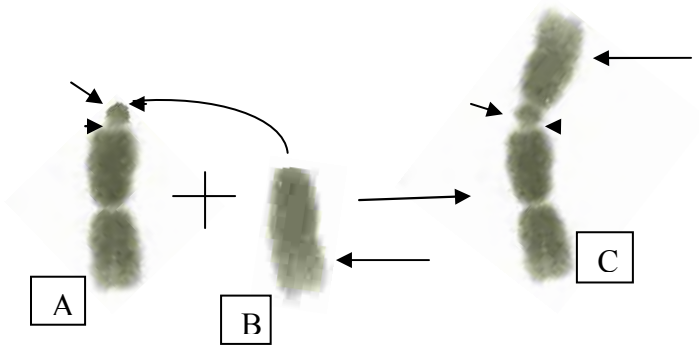


Figure 12 Possible origin of satellite chromosome and reduction of chromosome number in *K. insignis*. Suppose A is the original satellite chromosome (arrow head = secondary constriction, arrow = original satellite) and B is a translocation segment. If B is tranlocated on to A terminally, it would result in the present satellite chromosome of *K. insignis* (C). Loss of the chromosome that donated the translocated segment would reduce the chromosome number from 2n = 24 to 2n = 22.

Regarding the genome size, which was estimated as total length of a metaphase chromosome complement, the diploid species have genomes of comparable sizes, with *K. isoetifolia* having a slightly larger genome (Table 4). Owing to its polyploid nature, *K. insignis* has the largest genome, but this is less than double that of the diploid species. This can be interpreted as either one or both of the diploid parental species had genome smaller than that of the diploid species considered in the present study or genome downsizing might have occurred after polyploidization, which is a wide spread phenomenon in polyploids (Otto and Whitton, 2000). Generally, genome size estimates from chromosome length measurements give only a rough value because it is affected by factors such as differential degree of chromosome condensation.

The chromosome size of the investigated endemic *Kniphofia* species decreases gradually from the largest to the smallest chromosome in the complement, but there is only a narrow range between the largest and smallest chromosomes. This data strongly agree with the report of Tilahun Teklehaymanot (2001) on endemic species.

Excluding *K. insignis*, there is no significant differentiation in the arm ratios between the corresponding pairs of chromosomes of the studied (diploid) species. As could be seen from

the karyotypes (Figures 2, 8, 5) and the r values (Table 5), in three of the diploid species (*K. foliosa*, *K. hildebrandtii* and *K. schimperi*) chromosome pair I is m type, pairs II, III, V and VI are sm type and pair IV is st type. Eventhough pair IV in *K. isoetifolia* falls in sm group, relatively, it has the highest arm ratio in the group which make it close to pair IV of the other diploid species. Furthermore, pair II of *K. isoetifolia* is on the boarderline between m and sm, thus having arm ratios close to that of pair II of the other diploid species. Thus, the former three species have karyotype formula of 2m + 8sm + 2st, whereas the latter species has 2m + 2m/sm + 8sm.

The chromosome size of the investigated species showed slight variation within and among species. Within the diploid species, there is no large difference in relative chromosome size. In each case, the shortest chromosome is more than 70% as long as the longest chromosome in the complement. This shows that, regarding the relative chromosome size, the karyotypes show weak asymmetry. Thus, the asymmetry of the karyotypes is largely based on the centromeric position with most of the chromosome having submedian and subterminal centromer. The size range between the largest and smallest chromosomes is wider for *K. insignis* in which the smallest chromosome is only about 45% as long as the longest pair in the complement. However, the size range between chromosomes of pair 2 to 7 is very narrow as that of between pairs 8 to 12, which allows the grouping of the ten pairs into two size classes.

In the present study, it was found that satellites occur at similar positions (terminal) in all the diploid species, but the number observed varies between species. It needs further study to determine if the observed differences between different species are genuine or due to their escaping of detection owing to various factors such as their small size.

In the present study, *K. foliosa* specimens collected from ‘Laga Shore, ca 10 km from Gedo on the road to Fincha Sugar Factory contain a B-chromosome in the root tip cells. This is the first report for Ethiopian endemic *Kniphofia* species. The B-chromosome is metacentric (m) and shows a normal disjunction during mitosis (Figure 4). No more than 1B was observed in individual cells examined, which implies that the B-chromosome has normal anaphase disjunction. However, it would be interesting to observe different tissues or organs of the

plants, other than the root tip, to see if the B chromosome disjoins regularly in the other tissues as well. It would also be interesting to know how the B-chromosome behaves during meiosis when one or more of them are present in a cell and what mechanism it employs to counteract elimination. Unfortunately, meiotic study could not be done in the present study as the material did not flower in the greenhouse during the study period.

Generally, the karyotype evolution of the studied species clearly indicated that there is stability among four endemic species (*K. foliosa, K. schimperi, K. hildebrandtii,* and *K. isoetifolia*) as far as the chromosome number 2n = 12 is concerned and this stability is shared among different species in South Africa which is considered to be the homeland for *Kniphofia* (Moffett, 1930). However, the polyploid case discovered in *K. insignis* in the present study clearly indicates that, gradual evolution is taking place among the endemic *Kniphofia* species with respect to chromosome number and morphology.

A point that would be worthy of consideration is the taxonomic status of the polyploid specimens found in the present study. Do these specimens represent a new chromosomal race of *K. insignis* which, till now is known only as diploid, or do they represent a new species (biological or otherwise) of the genus *Kniphofia*. These and the question of possible diploid progenitors of this polyploid will remain for future investigation.

6. **Conclusions and Recommendation**

6.1. Conclusion

The present study determined and confirmed the chromosome number, karyotype, karyotype formula and ploidy levels of five endemic *Kniphofia* species of Ethiopia. The result obtained showed that four of the five studied species (*K. foliosa, K. schimperi, K. hildebrandtii, and K. isoetifolia*) have a diploid chromosome number of 2n = 12 with the basic chromosome number of (x = 6). One endemic species (*K. insignis*) was found to be polyploid (tetraploid) with a chromosome number of 2n = 22, which is assumed to be a result of aneuploid reduction on the basis of x = 6 basic chromosome number of the genus. B-chromosome has been recorded in one of the species, *K. foliosa*.

In the study, it was also obtained that four species: *K. foliosa, K. schimperi, K. hildebrandtii* and *K. isoetifolia* possess satellites on the tips of the short arms while *K. insignis* showed a large secondary constriction at the middle of the long arm in one pair of the chromosomes and has the largest satellite of all the species studied.

The distinctness in chromosome number and chromosome morphology observed in *K. insignis* rules out any possible speculation that the specimens studied are just happened to be rare mutations in the population in which the chromosome number has been doubled. The karyotype of this species is quite distinct from any of the karyotypes described in this study.

The karyotype of the studied species is more or less unimodal since the chromosomes show a gradual decrease in size with no formation of distinct classes of chromosomes with regard to their relative sizes.

The present study has shown that the chromosomes of all the species show weak differentiation with regard to their relative sizes, but they show strong asymmetry with regard to the centromeric positions in which the majority of the chromosomes of each species are sm, as is evident from the karyotype formula of 2m + 8sm + 2st for *K. foliosa, K. schimperi*, and *K. hildebrandtii,* 2m + 2m/sm + 8sm for *K. isoetifolia* and 4m + 10sm + 2 m/st + 6st for *K.insignis*.

6.2. Recommendations

Based up on the results, the following points are recommended

1. Further study should be carried out on *K. insignis* by including more samples from different localities in order to establish the extent, distribution and nature of the polyploidy.
2. In order to have well sounding results on cytogenetical study, further study including the chromosome behavior during meiosis and pollen fertility in interspecific hybrids should be included.
3. Techniques like C-banding and silver staining should be used to reveal more information.
4. More advanced molecular cytogenetic techniques such as FISH and GISH should be carried out to reveal more information about the chromosomes of *Kniphofia* species.
5. In order to better understand the genetic diversity and phylogenetic relationship among *Kniphofia* species, biochemical and molecular studies are recommended

7. **References**

Abrham, Abebe Wube, Bucer, F., Kaleab Asres, Gibson, S., Rattray, L., and Croft, L.S. (2005). Antimalarial compounds from *Kniphofia foliosa* roots. *Phytother. Res*. **19**: 472-476.

Anne-Lutz (1916). *Oenothera* mutants with diminutive chromosomes. *Ame. J. Bot.* **3**(9): 502-526.

APG (Angiosperm Phylogeny Group). (1998). An ordinal classification for the families of flowering plants. *Ann. Missouri Bot. Gard.* **85**: 531-553.

APG (Angiosperm Phylogeny Group). (2003). An update to the Angiosperm Phylogeny Group classification for the orders and families of the flowering plants: APG II. *Bot. J. Linne. Soc.* **141**: 399-436.

Battaglia, E. (1964). Cytogenetics of B chromosomes. *Caryologia* 17: 245-249.

Beaumont, J., Cutler, D.F., Reynolds, T. and Vaughan, J.G. (1985). The secretary tissue of aloes and their allies. *Israel J. Bot.* **34**: 265-282.

Bennet, M.D. (1998). Plant genome values: how much do we now? *Proc. Natl. Sci. U.S.A*. **95**: 2011-2016.

Berger, A. (1908). Liliaceae-Asphodeloideae-Aloineae. In: *Das Pflanzenreich, ed. Engler, A*.,Vol. 30. Engelmann, Leipzig.

Bringmann, G., Menche, D., Bezabih, M., Birhanu Abegaz Molla. and Kaminsky, R. (1999). Antiplasmodial activity of Knipholone and related natural phenylanthraquinones. *Planta Medica* **65**: 757-758.

Burt, A., and Trivers, R. (1998). Selfish DNA and breeding system in flowering plants. *Biological Sciences* **265**: 141-146.

Camacho, J.P.M, and Parker, J. (1994). Structural and functional evidence that a B-chromosomes in the Characid fish *Astyanax scabripinnis* is an isochromosome. *Heredity* **85**: 1-9.

Camacho, J.P.M., Sharbel, T.F., and Beukeboom, L.W. (2000). B chromosome evolution. *Biological Sciences* 355: 163-178.

Chase, M.W., Duvall, M.R., Hills, H.G., Conran, J.G., Cox, A.V., Eguiarte, L.E., Hartwell, J., Faye, M.F., Caddick, L.R., Cameron, K.M., & Hoot, S. (1995). Molecular phylogenetics of Lilianae. In: *Monocots: Systematics and Evolution*, eds.

Cheng, Z.K., Yu, H.X., Yan, H.H., Gu, M.H., and Zhu, L.H. (2000). B chromosome in a rice aneuploid variation. *Theo. App. Genet*. **101**: 564-568.

Codd, L.E. (1968). The South African species of *Kniphofia*. *Bothalia* **9**(3&4): 363-513.

Cremer, T and Cremer, C. (1998). Chromosomes in focus: Basic cytogenetics, light microscopy and the case of Neotropical Fish. *Cytogenetics and Cell Genetics*, **48**: 66.

Cronquist, A. (1981). *An Integrated System of Classification of Flowering Plants*. British Columbia Press, New York. Pp. 1263.

Dahlgren R, Clifford, H.T and Yeo, P.F. (1985). *The Families of the M*onocotyledons*,Structure, Evolution and Taxonomy*. Springer-Verlag, Berlin. Pp. 520.

De Melo, N.F., Cervi, A.C. and Guerra, M. (2001). Karyology and cytotaxonomy of the genus *Passiflora* L. (*Passifloraceae*). *Pl. Syst. Evol.* **226**: 69-84

de Wet, J.M.J. (1960). Chromosome morphology in *Kniphofia. Bothalia* **7**:295-297.

de Wet, J. M. J. (1971). Reversible tetraploidy as an evolutionary mechanism**.** *Evol.* **25** (3): 545-548.

Dhar, M.K., Friebe, B., Koul, A.K., and Gill, B.S. (2002). Origin of an apparent B chromosome by mutation, chromosome fragmentation and specific DNA sequence amplification. *Chromosoma* **111**: 332-340.

Elovson, S. (1984). *Wild Flowers of Southern Africa*, 7th edn. Pretoria, South Africa.

Ermias Dagne and Steglich, W. (1984). Knipholone, a unique anthraquinone derivative from *K. foliosa*. *Phytochemistry* **23**: 1729-1731.

Esayas Berhanu, Masresha Fetene, and Ermias Dagne. (1986). Athraquinones as taxonomic markers in Ethiopian *Kniphofia* species. *Phytochemistry* **25:** 847-850.

Evans, G.M., and Davies, E.W. (1985). The genetics of meiotic chromosome pairing in *Lolium temulentum* X *Lolium perenne* tetraploids. *Theo. App. Genet*. **71**: 185-192.

Fichtl, R. and Admassu Adi. (1994). *Honeybee Flora of Ethiopia*. DED, Margeaf Verlang. Pp. 510.

Gonzalez-Sanchez, M., Chiavarino, M., Jimenez, G., Manzanero, S., Rosato, M., and Puertas, M.J. (2004). The parasitic effects of rye B chromosomes might be beneficial in the long term. *Cytogen. Geno. Res.* **106**: 386-393.

Grant, V. (1981). *Plant Speciation*. Second edition. Colombia Univ. Press. New York. Pp. 563.

Green, D.M. (1990). Muller Ratchet and the evolution of supernumerary chromosomes. *Genome* 33: 818-824.

Greilhuber, J. and Ehrendorfer, F. (1988). Karyological approaches to plant taxonomy. *Pl. and Ani. Sci.* **376**: 289-297.

Gupta, K. (2004). *Cell and Molecular Biology*. 3rd ed. Rastogi Publication, New Delhi, India.

Hilton-Taylor, C. (1996). Red Data List of southern African Plants. *Strelitzia* **4**: 1-117.

Hiremath, S.C., Murthy, H.N. and Salimath, S.S. (1992). Quantitative nuclear DNA differences associated with genome evolution in *Guizotia* (Compositae). *Genetica* **85**: 241-247.

Holmquist, G. and Dancis, B.M. (1980). A general model of karyotype evolution. *Genetica* **53**: 151-163.

http//www.plantzafrica.com/plantklm/**kniphofias**.htm *Kniphofia* Moench: family asphodelaceae Common names: Red-hot poker or Torch lily (English), Vuurpyl (Afrikaans) Accesed on July 10/2008

Jackson, R.C. (1971). The Karyotype in Systematics. *Ann. Rev. Ecol. Syste.* **2**: 327-368.

Janaki-Amal, E.K. (1950). A triploid *Kniphofia*. *J. Roy. Hort.Soc*. **75**: 23-30.

Jenkins, G. (1986). Synaptonemal complex formation in hybrids of *Lolium temulentum* X *Lolium perenne* (L). 3. Tetraploid. *Chromosoma* **93:** 413-419.

Jones, N., and Houben, A. (2003). B chromosomes in plants: escapees from the chromosome genome? *Tren. P. Sci.* **8:** 417-423.

Kativu, S. (1996). Asphodelaceae of the Flora Zambesiaca area. *Kirkia* **16**: 27-53.

Kifle Dagne (1995). Mitotic and meiotic behavior of B-chromosomes of *Guizotia scabra* (Vis) chlov.sp Scabra (Compositae). *Caryologia*, **48:** 35-45.

Kifle Dagne (1994). Meiosis in interspecific hybrids and genomic interrelationships in *Guizota abyssinica* (Compositae). *Hereditas* **121**: 119-129.

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

Lavania, S**.** and Srivastava U.C., (1992). Genetic variation in meiotic behaviour and fertility in tetraploid *Hyoscyamus muticus*. *J. Plant Syst. Evol*. **11**: 112-136.

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

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

Levin, D.A., Palestis, B.G., Jones, R.N., and Trivers, R. (2005). Phyletic hot spots for B chromosomes in angiosperms. *Evolution* **59**: 962-969.

Levitzky, G. A. (1931). The karyotype in systematics. *Trudy. Prikl. Bot*. **27**: 220-240.

Lewis, W. (1980). *Polyploidy*. *Biological Relevance.* New York. Pp. 199-214.

Lia, V.V., Confalonieri, V.A., and Poggio, L. (2007). B chromosome polymorphism in maize landraces: Adaptive vs. demographic hypothesis of clinal variation. *Genetics* **177**: 895-904.

Mable, B.K. (2004). Why polyploidy is rarer in animals than in plants: myths and mechanisms. *Biol. J. Linn. Soc*. **82**: 453–466.

Marais, W. (1973). A revision of the tropical species of *Kniphofia* (Liliaceae). *Kew Bulletin* **28**: 465-483.

Moffett, A.A. (1930). Studies on the formation of multinuclear giant pollen grains in *Kniphofia*. John Innes Horticultural Institution*, Merton.* No volume pp.315-337.

Muntzing, A., and Nygren, A. (1955). A new diploid variety of *Poa alpina* with 2 accessory chromosomes at meiosis. *Hereditas* **41**: 405-410.

Nayak, S. and Sen, S. (1992). Karyological and cytophotometric study of explant drived clones on non polysomatic and poysomatic species of *Kniphofia*. *Biologia Plantarum* **34**: 135-141.

Neo, D.M., Filho, O.M., and Camacho, J.P. (2000). Altitudinal variation for B chromosome frequency in the characid fish *Astyanax scabripinnis*. *Heredity* **85**: 136-141.

Otto, S.P and Whitton, J., (2000). Polyploid incidence and evolution. *Annu. Rev. Genet*. **34**: 401–37.

Plowman, A.B., and Bougourd, S.M. (1994). Selectively advantageous effects of B chromosomes on germination behavior in *Allium schoenoprasum* L. *Heredity* **72**: 587- 593.

Ramdhani, S. (2007). Evolutionary and Biogeographic Studies in the genus *Kniphofia* Moench (Asphodelaceae) Ph.D thesis, Botany Department, Rhodes University. Pp. 410.

Ramdhani, S., Barker, N.P. and Baijnath, H. (2006). Phylogenetics of the genus *Kniphofia* Moench (Asphodelaceae). In: Taxonomy and Ecology of African Plants: Their Conservation and Sustainable Use (Proceedings of the 17th AETFAT Congress), eds. Ghazanfar, S.A. &Beentje, H.J., pp. 559-573. *Royal Botanic Gardens*, *Kew.*

Ramsey, J., and Schemske, D.W. (2002). Neopolyploidy in flowering plants. *Ann. Rev. Eco. Syst*. **33**: 589–639.

Randolph, L.F. (1928). Types of supernumerary chromosomes in *Sorghum nitidupers*. *J. Ind. Soci*. **39:** 278-280.

Robertson, W. R. B. (1916). Chromosome studies I. Taxonomic relationships shown in the chromosomes of *Tettigidae* and *Acrididae*: V-shaped chromosomes and their significance in *Acrididae*, *Locustidae*, and *Gryllidae*: Chromosomes and variation. *J. Morphol*. **27**: 179-331.

Schmid, M., Ziegler, C.G., Steinlein, C., Nanda, I., and Haaf, T**.** (2002). Chromosome banding in amphibia - XXIV. The B chromosomes of *Gastrotheca espeletia* (Anura, Hylidae). *Cyto. Geno. Res.* **97:** 205-218.

Schubert, I., Rieger, R., and Künzel, G. (1991). *Chromosome Engineering in Plants: Genetics, Breeding, Evolution.* (Part A), (eds. Gupta, P. K. and Tsuchiya, T). Elsevier, Amsterdam, 113-152.

Scott-Shaw, R. (1999). *Rare and Threatened Plants of KwaZulu-Natal and Neighbouring Regions.* Teeanem Printers, Pietermaritzburg. Pp. 171-175.

Sebsebe Demissew and Nordal, I. (1997). Aspodelaceae. In: *Flora of Ethiopia and Eritrea* Vol. 6. Hydrocharitaceae to Arecaceae. Pp. 106-115. (Edwards, S., Sebsebe, D. and Hedberg, I., eds.) EMPDA. Addis Ababa, Ethiopia and Uppsala, Sweden.

Sharma, A.K. (1954). Cytology of some of the members of Commelinaceae and its bearing on the interpretation of phylogeny. *Genetica* **27**: 323-363.

Sharma, A.K. and Sharma, A. (1959). Chromosomal alterations in relation to speciation. *Bot. Rev*. **3**: 514-544.

Singh, R.J. (2003). *Plant Cytogenetics*. CRC Press. Boca Raton, London, New York.

Sinha, U. and Sinha, S. (1976). *Cytogenetics, Plant Breeding and Evolution*. Vikas Publishing House Pvt, Ltd. Delhi. Pp.60-61.

Smith, G.F. and van Wyk, A.E. (1992). Systematic leaf anatomy of selected genera of southern African Alooideae (Asphodelaceae). *Sou. Afr. J. Bot.* **58**: 349-359.

Smith, G.F. and van Wyk, B.E. (1998). Asphodelaceae. In; *The Families and Genera of Vascular Plants. III Flowering Plants; Monocotyledons. Lilianae* (except *Orchidaceae*). Pp.130-140. Springer. Berlin.

Soltis, D.E., Soltis, P.S., and Tate, J.A. (2003). Advances in the study of polyploidy in the plant species. *New Phytol*. **161**: 173-191.

Speicher, R.M. and Carter, P.N. (2005). The new cytogenetics: Blurring the boundaries with molecular biology. *Nat. Rev. Genet.* **6**: 782-792

Stace, C. (1980). *Plant Taxonomy and Biosystematics*. Edward Arnold, London. Pp. 272.

Stace, C.A. (1991). *Plant Taxonomy and Biosystematics*. 2nded. Cambridge University Press, Cambridge, 264pp.

Stace, C. (2000). Cytology and cytogenetics as a fundamental taxonomic resource for the 21st centuries. *Taxon*. **49**: 2011-2016.

Staub, R.W**.** (1987). Leaf striping correlated with the presence of B chromosomes in maize. *J. Heredity* **78:** 71-74.

Stebbins, G. L., (1971). *Chromosomal Evolution in Higher Plants*. Edward Arnold (Publishers) Ltd., London. Pp. 216.

Stevens, N.M. (1908). The chromosomes in *Diabrotica vittata, Diabrotica soror and Diabrotica* 12-*punctata*. A contribution to the literature on heterochromosomes and sex determination . *J. Exp. Zolo*. **5**: 453-470.

Tilahun Teklehaymanot (2001). Distribution, reproductive biology, demography and genetic diversity of endemic and indigenous *Kniphofia* species in Ethiopia. Ph.D thesis, Addis Ababa University, Addis Ababa. Pp. 119.

Tilahun Teklehaymanot, Endashaw Bekele, Sebsebe Demissew, and Nordal, I. (2004). Isoenzyme analysis of five endemic and one wide spread *Kniphofia* species (Asphodelaceae) of Ethiopia. *SINET. Ethiop. J. Sci*. **27**(2): 143-152.

van Staden, L.F. and Drewes, S.E. (1994). Knipholone from *Bulbine latifolia* and *Bulbine frutescens*. *Phytochemistry* **35**: 685-686.

van Wyk, B.E., Yenesew Abebe and Ermias Dagne. (1995). Chemotaxonomic significance of anthraquinones in the roots of asphodeloideae (Asphodelaceae). *Bioche. Syst. Ecol.* **23**: 277-281.

Webber, J.M. (1932). Chromosome morphology and meiotic behaviour in typical and variant forms of *Kniphofia aloides*. *Amer. J. Bot*. **15:** 411-422.

White, M. J. D. (1970). Heterozygosity and genetic polymorphism in parthenogenetic animals. *J. Evol. Genet*. **23**: 237-62.

Wu, T.P. (1992). B-Chromsomes in *Sorghum stipoideum*. *Heredity* **68**: 457-463.

Appendix 1: Chromosome measurements in (cm), arm ratios and position of centromers in *Kniphofia foliosa.*

Chr. Numb.	Chr. Length	Long arm	Short arm	Arm ratio (L/S)	Centr. Position
1	3.45	2.05	1.40	1.46	m
2	3.35	2.00	1.35	1.48	m
3	3.40	2.25	1.15	1.96	sm
4	3.33	2.20	1.10	2.00	sm
5	3.00	1.95	1.10	1.77	sm
6	3.15	2.00	1.15	1.74	sm
7	2.95	2.20	0.75	2.93	sm/st
8	2.80	2.15	0.65	3.31	st
9	2.75	1.95	0.80	2.44	sm
10	2.70	1.90	0.80	2.38	sm
11	2.55	1.80	0.75	2.40	sm
12	2.45	1.75	0.70	2.50	sm
13	0.55	0.30	0.25	1.20	m

Total chromosome length per diploid = 35.88 cm.

Appendix : Chromosome measurements in (cm), arm ratios and position of centromers in *Kniphofia schimperi.*

Chr. Numb.	Chr. Length	Long arm	Short arm	Arm ratio (L/S)	Centro. Position
1	3.60	2.15	1.45	1.48	m
2	3.35	2.00	1.35	1.48	m
3	3.15	2.05	1.10	1.86	sm
4	3.20	2.05	1.15	1.78	sm
5	2.96	2.10	0.86	2.44	sm
6	2.80	2.10	0.70	3.00	sm/st
7	2.80	2.15	0.65	3.31	st
8	2.55	1.90	0.65	2.92	sm
9	2.60	1.90	0.70	2.71	sm
10	2.95	2.10	0.85	2.47	sm
11	2.60	1.85	0.75	2.47	sm
12	2.50	1.80	0.70	2.57	sm

Total chromosome length per diploid = 35.06 cm.

Appendix 3: Chromosome measurements in (cm), arm ratios and position of centromers in *Kniphofia hildebrandtii.*

Chr. Numb.	Chr. Length	Long arm	Short arm	Arm ratio (L/S)	Centr. Position
1	3.20	1.70	1.50	1.13	m
2	3.20	1.95	1.25	1.56	m
3	3.25	2.05	1.20	1.71	sm
4	3.05	2.00	1.05	1.90	sm
5	2.95	2.00	0.95	2.11	sm
6	2.85	1.95	0.90	2.17	sm
7	2.80	2.15	0.65	3.31	st
8	2.85	2.15	0.70	3.07	st
9	2.65	1.95	0.70	2.79	sm
10	2.80	2.05	0.75	2.73	sm
11	2.45	1.80	1.65	2.77	sm
12	2.40	1.75	0.65	2.69	sm

Total chromosome length per diploid = 34.45 cm

Appendix 4: Chromosome measurements in (cm), arm ratios and position of centromers in *Kniphofia isoetifolia.*

Chr. Numb.	Chrom. Size	Long arm	Short arm	Arm ratio (L/S)	Centrom. Position
1	3.95	2.30	1.65	1.39	m
2	3.85	2.30	1.55	1.48	m
3	3.60	2.25	1.35	1.67	m/sm
4	3.60	2.25	1.35	1.67	m/sm
5	3.30	2.25	1.05	2.14	sm
6	3.40	2.40	1.00	2.40	sm
7	3.15	2.15	1.00	2.15	sm
8	3.10	2.25	0.85	2.65	sm
9	2.95	2.10	0.85	2.47	sm
10	3.30	2.35	0.95	2.47	sm
11	3.05	2.20	0.85	2.59	sm
12	2.70	1.90	0.80	2.38	sm

Total chromosome length per diploid = 39.95 cm.

Appendix 5: Chromosome measurements in (cm), arm ratios and position of centromers in *Kniphofia insignis.*

Chr. Num.	Chr. Length	Long arm	Short arm	Arm ratio (L/S)	Centro. Position
1	4.10	2.30	1.80	1.28	m
2	4.00	2.25	1.75	1.29	m
3	2.95	2.05	0.90	2.28	sm
4	2.95	2.10	0.85	2.47	sm
5	2.85	2.15	0.70	3.07	st
6	2.80	2.10	0.70	3.00	sm/st
7	2.70	2.10	0.60	3.50	st
8	2.80	2.15	0.65	3.31	st
9	2.50	1.85	0.65	1.85	sm
10	2.75	2.00	0.75	2.67	sm
11	2.75	2.10	0.65	3.23	st
12	2.45	1.90	0.55	3.46	st
13	2.50	1.85	0.65	2.85	sm
14	2.50	1.90	0.60	3.17	st
15	1.85	1.30	0.55	2.36	sm
16	1.90	1.30	0.60	2.17	sm
17	1.75	1.20	0.55	2.18	sm
18	1.75	1.20	0.55	2.18	sm
19	1.85	1.20	0.65	1.85	sm
20	1.75	1.15	0.65	1.77	sm
21	1.90	1.05	0.85	1.24	m
22	1.80	1.10	0.7	1.57	m

Total chromosome length per tetraploid = 55.15 cm.

Declaration

I, the undersigned, declare that this thesis is my original work. It has never been submitted and presented in any institution and that all sources of materials used for the thesis have been duly acknowledged.

Name: **Fekadu Gadissa**

Signature: ----------

Date: ----------

Approved by:

Name: ----------

Signature: ----------

Date: ----------