Addis Ababa University
School of Graduate Studies
Department of Biology

CHROMOSOME STUDY OF SOME ETHIOPIAN ANURAN AMPHIBIANS

A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology (Applied Genetics)

By

Andualem Tsige

July 2008.
Addis Ababa

6. DISCUSSION.....41
7. CONCLUSIONS AND RECOMMENDATIONS.....46
7.1. CONCLUSIONS.....46
7.2. RECOMMENDATIONS.....47
REFERENCES.....48
APPENDICES.....54

ACKNOWLEDGEMENT

In presenting this thesis, I very much thank my God for all His blessings to me and above all for all good things that He has done to me throughout my life as well as during this study.

I am very much honoured of expressing my very special acknowledgment to my academic advisor and research supervisor Dr. Kifle Dagne for all his unlimited commitment and countless professionally pertinent supports to successful accomplishment of my research and thesis works. Truly, his genuine and valuable advice, critics and comments as well as unreserved guidance throughout the study are highly appreciated; of course are unforgettable. Thanks and appreciations should go to my co-advisor Prof. Samy A. Saber for his professional help in identification of amphibian specimens.

I wish to acknowledge the assistance and cooperation given to me at different stages of my study by the following friends: Solomon Salhi, Fisseha Ayele, Ermias Assefa, Mohammed Hussein, Belay Assefa, Girma Bogale, and Netsanet Bekele. Ambachew Ayalew has also supported my work since the beginning. His contribution is highly appreciated and gratefully acknowledged.

Thanks and appreciations are extended to Amhara National Regional State Education Bureau for sponsoring my graduate studies at Addis Ababa University. In addition, I also thank Biology Department of Addis Ababa University for accepting and training me as a postgraduate student.

Part of the costs of this thesis research was covered by Amphibian Diversity Project of the Natural History Museum, Department of Biology, Science Faculty of Addis Ababa University through Dr. Abebe Getahun. Therefore, thanks and appreciations are extended to this Project and Dr. Abebe Getahun.

Finally, I obtained valuable cooperation and assistance from many people. I regret that only a few are mentioned here. However, to all I extend my sincere thanks.

LIST OF TABLES **Page**

Table 1. Living anuran families and common names with number of genra according to Myers, *et al*., 2006**.** ----------6

Table 2. Ethiopian amphibian families according to Largen (2001). ----------9

Table 3. Species of amphibians studied, their geographic locations and number of amphibians captured for present study ----------24

Table 4. Karyological data of the studied amphibians from different localities in Ethiopia showing the different centromeric positions, the diploid and fundamental numbers ----------40

LIST OF FIGURES **Page**

Figure 1. Map of Ethiopia showing the sites of specimen collection ----------23

Figure 2. Pictures of specimens captured from different localities in Ethiopia ----25

Figure 3. Somatic metaphase chromosome spread of *Rana angolensis* specimens from Addis Ababa ----------30

Figure 4. Karyotypes of *Rana angolensis* specimens from Addis Ababa ----------31

Figure 5. Somatic metaphase chromosome spread and Karyotype of male *Xenopus clivii* ----------32

Figure 6. Somatic metaphase chromosome spread and Karyotype of *Hyperolius* specimens from Addis Ababa ----------33

Figure 7. Somatic metaphase chromosome spread of *Ptychadena neumanni*; (A) Male specimen from Ziway (Z-L1), (B) Female specimen from Ziway (Z-L2), and (C) Male specimen from Dessie-Tenta (D-T5) ------34

Figure 8. Karyotypes of *Ptychadena neumanni* specimens; (A) Male from Ziway (Z-L1), (B) Female from Ziway (Z-L2), and (C) Male specimen from Dessie Tenta (D-T5) ----------35

Figure 9. Somatic metaphase chromosome spread and Karyotypes of female *Ptychadena cooperi* ----------36

Figure 10. Somatic metaphase chromosome spread of *Ptychadena anchiet* specimen from Goro ----------37

Figure 11**.** Somatic metaphase chromosome spread of *Bufo garmani* specimens from Debre Zeit (A) Female, and (B) Male ----------38

Figure 12. Karyotypes of *Bufo garmani* from Debre Zeit (A) Female, and (B) Male specimens ----------38

Figure 13. Somatic metaphase chromosome spread and Karyotypes of female *Bufo regularis* specimen from Debre Zeit. ----------39

LIST OF APPENDICES **Page**

Appendix 1. Zoogeographic affinities of the Ethiopian amphibians and their altitudinal ranges within which they have been recorded in Ethiopia----54

Appendix 2. Summary of sites of capture, number of male and females, number of metaphase plates studied ----------57

Appendix. 3 Analysis of haploid chromosome complement of karyotyped species -58

Appendix 4. Chromosome measurement: Total length of each chromosome, arm lengths, arm ratio, centromeric position and normalized length of *Rana angolensis* (Male specimen) ----------59

Appendix 5. Chromosome measurement: Total length of each chromosome), arm lengths, arm ratio, centromeric position and normalized length of *Rana angolensis* (Female specimen) ----------60

Appendix 6. Chromosome measurement: Total length of each chromosome, arm lengths, arm ratio, centromeric position and normalized length of *Ptychadena neumanni* (Male specimen) ----------61

Appendix 7. Chromosome measurement: Total length of each chromosome, arm lengths, arm ratio, centromeric position and normalized length of *Ptychadena neumanni* (Female specimen) ----------62

Appendix 8. Chromosome measurement: Total length of each chromosome, arm lengths, arm ratio, centromeric position and normalized length of *Ptychadena cooperi* (Female specimen) ----------63

Appendix 9. Chromosome measurement: Total length of each chromosome, arm lengths, arm ratio, centromeric position and normalized length of *Bufogarmani* (Male specimen) ----------64

Appendix 10. Chromosome measurement: Total length of each chromosome, arm lengths, arm ratio, centromeric position and normalized length of *Bufo garmani* (Female specimen) ----------65

Appendix 11. Chromosome measurement: total length of each chromosome, arm lengths, arm ratio, centromeric position and normalized length of *Bufo regularis* (Female specimen) ----------66

Appendix 12. Chromosome measurement: Total length of each chromosome, arm lengths, arm ratio, centromeric position and normalized length of *Hyperolius* spp. ----------67

Appendix 13. Chromosome measurement: Total length of each chromosome, arm lengths, arm ratio, centromeric position and normalized length of *Xenopus clivii* (Male specimen) ----------68

LIST OF ABBREVIATIONS

A-ST $_{(1)}$ = Addis Ababa - Selame Technical and Vocational Training Centre, specimen number 1

A-AF $_{(1)}$ = Addis Ababa - Abyssinia Tea and Coffea Factory, specimen number 1

A-EK $_{(3)}$ = Addis Ababa - Entoto Kidane Mhiret Church, specimen number 3

A-EK$_{(10)}$ = Addis Ababa - Entoto Kidane Mhiret Church, specimen number 10

A-K$_{(2)}$ = Addis Ababa - Kotebae, specimen number 2

D-T$_{(5)}$ = Dessie - Tenta, specimen number 5

DZ-C$_{(2)}$ = Debre Zeit - Lake Cheleklaka, specimen number 2

DZ-C$_{(8)}$ = Debre Zeit - Lake Cheleklaka, specimen number 8

DZ-C$_{(11)}$ = Debre Zeit - Lake Cheleklaka, specimen number 11

Z-L$_{(2)}$ = Ziway - Lake Ziway, specimen number 2

Abstract

Eight families consisting of 24 genera and 63 species of amphibians have been recorded in Ethiopia, and out of these 40% are endemic. Little is dealt about the ecology and behaviour of amphibians, and karyotypes of amphibians in Ethiopia had not been studied prior to this study. Chromosome numbers and karyotypes are reported for eight species of anuran amphibians belonging to five genera, collected from various geographical locations in Ethiopia. Chromosome preparations were made from intestinal epithelial cells after pre-treatment with colchicin. Air-dried slide preparation was made by splashing techniques after treating the cells with 0.075 M KCL hypotonic solution for about 25 - 30 minutes followed by several rounds of centrifugation in fixative (3 methanol: 1 acetic acid). Chromosome characteristics were determined from mitotic metaphase chromosome spreads following chromosome staining with Giemsa in phosphate buffer (pH = 6.8). The result showed that four species: *Ptychadena anchietae* (Bocage 1867)*, Ptychadena neumanni* (Ahl 1924)*, Ptychadena cooperi* (Parker 1930)*, Hyperolius*; have 2n = 24; two species *Bufo*, *Bufo garmani* Meek 1897, and *B. regularis* Reuss 1834, have 2n = 20; one species of *Rana*; *Rana angolensis* Bocage 1866 has 2n = 26; and a species of *Xenopus*, *Xenopus clivii* Peracca 1898 has 2n = 36. All species under the study are dominantly bimodal karyotype, i.e., composed of distinctly large and small chromosomes, except *Xenopus clivii* that varies in a gradual manner with most of sm and st chromosomes of the set. In the present study, different species of a given genera have very similar karyotype so that it is impossible to differentiate the species only on the basis of their karyotype.

Key word: Asymmetrical, Chromosome, Cytogenetic, Karyotype, and Symmetrical.

1. INTRODUCTION

Amphibians are cold-blooded vertebrates possessing a soft, moist skin rich in glands that keep it moist; if scale is present (in the case of salamander), it is hidden in the skin. Except for limbless caecilians, amphibians have paired limbs as adult (Price, 2003). In the development of amphibians, eggs are usually layed in water where larvae (tadpole) develop until metamorphosis. Prior to metamorphosis, legs develop ready to take over locomotion as the tail is absorbed and numerous changes occur in internal and external anatomy during transformation (Mertens, 1960).

The word amphibian reflects the double mode of life of amphibians that, they usually spend part of their lives in the water and part on land. Most amphibians such as the clawed frogs (*Xenopus*) spend most of their lives in water and leaving water only for short migration or hibernation. On the other hand, amphibians like the bush squeakers (*Arthroleptis*) spend their entire lives out of water. Eggs of these amphibians are laid in moist subterranean holes or rotting wood and the larval period is completed in the egg before hatching (Stewart, 1967).

Respiratory strategies also vary widely among amphibians. Larval forms breathe via gills while adult forms may have gills or primitive lungs. Among gilled adult forms, the gills may be external or internal. Some salamanders have neither lungs nor gills and respire transcutaneously. Even when other respiratory structures are present, the skin is the principal respiratory organ for most amphibians. Therefore, it is important that they be able to keep the skin moist (Price, 2003).

Contrary to the thinking of most people, amphibians are the most gentle and the safest to handle than all groups of vertebrates. Most of them are completely harmless; however, some toads and a few frogs may produce secretion as one of their protective mechanisms, which may irritate the skin of some people. Besides their harmless nature, most amphibians have medicinal and commercial importance. In addition, some amphibians have been used as model organism for various biological researches. However, man has exercised a deep

and lasting influence on the natural distribution of amphibians (Stewart, 1967).

According to the current view of vertebrate evolution, amphibian originated in late Devonian period 280 million years ago from crossopterygian fish. They are classified into two extinct subclasses, Labyrinthodontia and Lepospondyli, and one living subclass Lissamphibia. Living amphibians are classified into three orders and over 6000 species (Vences, *et al.,* 2006; Frost, 2007). Two of the present well known orders of amphibians are the newts and salamanders, which form the tailed amphibian or urodeles (caudate), and the frogs and toads which are tailless amphibian or Anura (salientia). Urodeles are few in number of species as compared to the anurans. The third order of recent amphibian is worm-like, limbless caecilians (Gymnophiona), some of them have small calcareous scales under the skin and are fewer in number than the other orders of amphibians (Mertens, 1960). As described in Largen (2001), eight families consisting of 24 genera and 63 species of amphibians have been recorded in Ethiopia, and out of these 40% are endemic (Appendix 1).

Systematic Zoologist makes use of different morphological character in working out the differences and relationships of various groups of animals. Amphibians, which are morphologically indistinguishable while biologically distinct, can be distinguished on the basis of their karyotypes (Sharma, 1991). Chromosomes can be studied in-terms of their microscopically visible size, shape, number and behaviors during meiosis and mitosis as a morphological manifestation of the genome. Karyological approaches to systematics and taxonomy make use of the detailed information on homology relationships between karyotypes and various characters that chromosome sets provide (Sharma, 1991). Karyological analysis provides useful information about evolution of systematic position and phylogenetic diversification of some amphibians (Cuevas and Formas, 2003). The main objective of this study is to provide information on the diversity of some Ethiopian anuran amphibians at chromosomal level.

2. LITERATURE REVIEW

2.1. Systematics of amphibians

Amphibians lack such definite ectodermal structures as hair, feathers, and scales; therefore taxonomist must rely on other characters. In addition to size, shape, and coloration, systematists make use of variation in the number of toes and of phalanges; the presence or absence of glands, especially the parotoid gland; the condition of the skin, whether smooth or rough. For many salamanders, the presence and number of costal grooves on the side of the body are useful characters (Orr, 1976).

Taxonomically, amphibians are divided into three subclasses: Subclass Labyrinthodontia (diverse Paleozoic and early Mesozoic group), Subclass Lepospondyli (small Paleozoic group), Subclass Lissamphibia (frogs, salamanders, etc). Subclass Lissamphibia, which includes all recent amphibians, is usually considered a clade (which means that it is thought that all Lissamphibians evolved from a common ancestor apart from other extinct groups, Labyrinthodontia and Lepospondyli). The Lissamphibia also traditionally subdivided into three orders: Order Gymnophiona or Apoda (caecilians), Order Caudata or Urodela (salamanders and newts), and Order Anura or Salientia (frogs and toads) (Amphibian; from Wikipedia, the Free Encyclopaedia; "http://en.wikipedia.org/wiki/Amphibian",).

The three orders of living amphibians are grouped into three orders, approximately 58 families, 389 genera, and more than 6000 species (Frost, 2007). However, these numbers fluctuate from author to author and from time to time due to new findings of species as well as merging of some species or genera together as a result of growing number of characters used in classification. The existing taxonomy of amphibians is based mainly on morphological characters. Ecological researches also provide clues to separates sibling species that were previously overlooked. At present, cytological and molecular techniques provide support for some arrangements based on morphological features (Beck and Slack, 2001).

Order Gymnophiona or caecilians are limbless, worm-like burrowing amphibians with poorly developed eyes and unusual characteristics such as small scales in the skin and well developed protrusible copulatory organs (Cogger, 1986). Although the caecilians are highly modified for a burrowing life, they retain many primitive features, and it seems certain that they originated from some other group of fossil amphibians than did the frogs and salamanders, presumably they arose from Lepospondyls (Noble, 1954). Caecilians are found in tropical areas of Africa, America, and Asia; but they are rarely seen owing to their burrowing habits. Order Gymnophiona are less diversed group than other orders with 160 known species (Schimid, *et al*., 1990). According to Myers, *et al*., (2006), order Gymnophiona, is classified into six families with 31 genera.

The order Caudata is composed of three extant suborders (Salamandroidea, Cryptobranchoidea, and Sirenoidea), and is further subdivided into nine families, some 62 genera, and more than 550 species and are mostly found in North America with only small number of species in Europe and Asia. But, for some unknown reason, they are not found in the southern hemisphere (Frost, 2007). The Salamandroidea, which contains approximately 90% of the Caudata members, has six families that accomplish "internal fertilization" by means of spermatophores. On the other hand, the Cryptobranchoidea (composed of the families Cryptobranchidae and Hynobiidae) and the Sirenoidea (composed of the family Sirenidae) contain species for which fertilization is external (Hasumi, 2003).

Order Caudate or urodela includes salamanders, newts and sirens. All urodeles possess tail, their body form is much like that of a lizard, and their larvae, if aquatic, resemble their parents closely, having, among other features, teeth in both jaws. The newts appear to be rather primitive amphibians because they have a rather simple shape. Their legs are rather weak and small, and that the body often partly drags along the ground when the animal is moving. They rely on lateral undulations of the body to assist the movement. But these primitive features are probably deceptive, and there is no evidence to suggest that they

arose any earlier than the other orders of modern amphibians. Sirens are completely aquatic, lack hind limbs and have external gills. Salamanders are long-tailed amphibians that usually have two pairs of limbs of approximately equal size (Larson, *et al*., 2006).

Anura is an order that includes frogs and toads. The use of the common names "frog" and "toad" has no taxonomic justification. From a taxonomic perspective, all members of the order Anura are frogs, but only members of the family Bufonidae are considered to be “true toads". The use of the term "frog" is a common name usually refers to species that are aquatic or semi-aquatic with smooth or moist skins, and the term "toad" generally refers to species that tend to be terrestrial with dry and warty skin. An exception is the Fire-bellied toad (*Bombina bombina*): while its skin is slightly warty, it prefers a watery habitat (Frog, From Wikipedia, the Free Encyclopaedia; Retrieved from "http://en. Wikipedia.org/ wiki/Frog", last modified 20:09, 2 June 2007).

There are around 5,280 species currently described in the order Anura. The living Anurans are typically divided into three suborders: Archaeobatrachia, which includes four families of primitive frogs; Mesobatrachia, which includes six families of more evolutionary intermediate frogs; and Neobatrachia, by far the largest group, which contains the remaining families of "modern" frogs, including most common species throughout the world (Table 1). This classification is based on such morphological features as the number of vertebrae, the structure of the pectoral girdle, and the morphology of tadpoles (Myers, *et al*., 2006). While this classification is largely accepted, relationships among families of frogs are still debated. The establishment of molecular genetics as a tool in systematics and the reconstruction of phylogenetic relationships have led to accumulation of many gene sequences of amphibians and the compilation of a complete phylogenetic tree of the amphibians has been proposed by initiatives such as AmphibiaTree (Vences, *et al*., 2006).

According to Largen (2001), 8 families consisting of 24 genera and 63 species of amphibians have been recorded in Ethiopia (Appendix 1). All are anurans, except one species (*Sylvacaecilia grandisonae*) of the order gymnophiona.

Table 1. Living anuran families and common names of anuran amphibians with number of genera according to Myers, *et al*., (2006)**.**

Suborder = Archaeobatrachia –4 families, 6 genera, 27 species		
Family	**No of Genera**	**Common Names**
Ascaphidae	1	Tailed frogs
Bombinatoridae	2	Fire-belly toads
Discoglossidae	2	Painted frogs or Disc-Tongued frogs
Leiopelmatidae	1	New Zealand primitive frogs
Suborder = Mesobatrachia - 6 families, 21 genera, 168 species		
Family	**No of Genera**	**Common Names**
Megophryidae	11	Litter frogs or short-legged toads
Pelobatidae	1	European spadefoot toads
Pelodytidae	1	Parsley frogs
Pipidae	5	Tongueless frogs or clawed frogs
Rhinophrynidae	1	Mexican Burrowing Toad
Scaphiopodidae	2	American spadefoot toads
Suborder = Neobatrachia - 21 families, more than 5,000 species		
Family (Some of the 21 families)	**No of Genera**	**Common Names**
Allophrynidae	1	Tukeit Hill Frog
Amphignathodontidae	2	Marsupial Frogs
Arthroleptidae	8	Screeching frogs or squeakers
Brachycephalidae	1	Saddleback toads
Bufonidae	35	True toads
Centrolenidae	3	Glass frogs
Dendrobatidae	9	Poison dart frogs
Heleophrynidae	1	Ghost frog
Hemisotidae	1	Shovelnose frogs
Hyperoliidae	20	Sedge frogs or bush frogs
Leptodactylidae	49	Southern frogs or tropical frogs
Mantellidae	5	= = =
Microhylidae	69	Narrow-mouthed frogs
Myobatrachidae	20	Australian ground frogs
Ranidae	52	True frogs
Rhacophoridae	9	Moss frogs
Rhinodermatidae	1	Darwin's frogs
Sooglossidae	2	Seychelles frogs

2.2. Geographical distribution and barrier of dispersal in amphibians

Amphibians are distributed on all continent of the world except Antarctic. They are unable to live in regions having permanently frozen subsoil. However, some amphibians such as *Rana* live North of the Arctic Circle in Norway and Russia; beside, one species of salamander (*Hynobius keyserlingii*) inhabits arctic Asia (Mertens, 1960). Even though amphibians are found in all areas where water is available, it is a well-known fact that geographical distribution shows variation among orders, families, genera and even to species level. In general, urodeles (Salamander and Newts) are found primarily in the northern hemisphere; North America has the greatest number of species, but they do not occur in Africa south of the Sahara. Caecilians are restricted to tropical regions; while the Anura (Frogs and Toads) are distributed over the entire world except in regions of extreme cold or aridity and they are most abundant in the tropics (Stewart, 1967).

Differences observed today in geographical distribution among various group of amphibians are due to their different times at which the groups arose in the past, mode of living, and the different barriers, which affect the dispersal of the group. Requirements of amphibian, which vary with the species, are so important in limiting their migration and dispersal. For example, *Ascaphus* succeed at temperatures few degrees above freezing and dies in captivity unless kept cool. The smooth-skinned *Bufo alvarius* can live successfully only near streams, while its rough-skinned relative *B. cognatus* is at home in the desert. All amphibians require some moisture, but as this requirement varies with the species, due to the inherited morphological and physiological peculiarities of the form, the moisture content of any one locality may determine the species living there (Noble, 1954).

As in the case of fishes and birds, the distribution of amphibian is often limited by their breeding-site preference. Frogs or salamanders, which lay their eggs near river streams usually, do not wander far from these locations. On high mountains, a large part of the frog fauna consists of species that skip over an aquatic larval stage and some salamanders that are ovoviviparous species. Similarly, in rain forests such as those in Jamaica, the frogs are species, which avoid an aquatic stage or forms with larvae adapted to survive in small

amounts of water caught between the leaves. In brief, both the restrictions of the habitat for breeding as well as the breeding preferences of the species have greatly influenced the present distribution of amphibians (Noble, 1954).

Man has exercised a deep and lasting influence on the distribution of amphibians. As mentioned in Mertens (1960), clearing of their original habitats for cultivation, destruction of virgin forests in all parts of the world and ruthless draining or poisoning of their native water have greatly restricted the natural distributional areas of many species and reduced their stocks.

Ethiopia is an important centre of biological diversity and endemicity of amphibians due to presence of land with huge environmental contrast. According to Largen (2001), there are 63 amphibian species recognized in Ethiopia. However, the total number surely increases when remote regions of the country are adequately surveyed. Most of these amphibians are savanna forms, distributed in lowland areas around the margins of the central plateau and usually widespread in other parts of Africa. Twenty-five species of these amphibians, which are distributed in seven of the eight families found in Ethiopia, are considered to be endemic (Table 2) and amongst which are six endemic genera: *Sylvacaecilia, Altiphrynoides, Spinophrynoides, Balebreviceps, Ericabatrachus and Paracassina.* Though three species (*Syluvacaecilia grandisonae, Leotoelis vannutellii* and *Afrixalus darkeorum*) appear to be confined to lowland forest in southwestern Ethiopia at altitudes below 2200m, the majority of endemics are most clearly associated with mountain forest or grassland in the altitudinal range of 1800-4000 m (Appendix 1).

Table 2. Ethiopian amphibian families according to Largen (2001).

FAMILY	ENDEMIC SPP.	TOTAL SPP.
Caeciliaidae	1	1
Pipidae	1	2
Bufonidae	3	13
Microhylidae	1	2
Ranidae	9	24
Rhacophoridae	-	1
Hyperoliidae	9	18
Hemisidae	1	2
Total species	25	63
% of total	39.7	

2.3. Importance of amphibians

Amphibians, especially anurans, are economically useful in reducing the number of insects that destroy crops or transmit diseases. Toads being indiscriminate feeders on insect life are valuable aids to the farmer in keeping down insect pests (i.e. act as biological control of insect pests). Frogs swarm in the paddy fields of Japan and China are undoubtedly useful in destroying obnoxious insects. In addition, frogs and toads are active in the evening and hence supplement the efforts of birds in retaining the balance of nature (Noble, 1954).

From the earliest times, human has hunted many of the large species of amphibians because they found their flesh palatable and because they need their fat. Notably, Europeans commercially exploit frogs as food; the French are the principal consumers, and they import thousands of tons of frog legs annually. Frog's legs form a staple article of food in various parts of the world. The principal species hunted for their legs are *Rana pipiens, R. catesbeiana*, and *R. palustris* (Mertens, 1960).

Dried toads and frogs are sold in China as food or for medical purposes. Toad skins have been used in Japan and elsewhere as a source of fine leather, besides in Japan dried salamanders are employed as a vermifuge (Noble, 1954).

Frogs have served as important model organisms throughout the history of science. Eighteenth-century biologist discovered the link between electricity and the nervous system through studying frogs. The African clawed frog, *Xenopus laevis*, was first widely used in laboratories in pregnancy assays in the first half of the 20th century. Frogs are also used in cloning research and other branches of embryology because frogs are among the closest living relatives of man to lack eggshells characteristic of most other vertebrates, and, therefore, facilitate observations of early development. Although alternative pregnancy assays have been developed, biologists continue to use *Xenopus* as a model organism in developmental biology because it is easy to rise in captivity and has a large and easily manipulatable embryo. Recently, *X. laevis* is increasingly being displaced by its smaller relative *X. tropicalis*, which reaches its reproductive age in five months rather than one to two years as in *X. laevis* (Beck and Slack, 2001).

Amphibians are excellent models to study sex differentiation, for a number of reasons. Genetic sex-determining systems and sex chromosomes have evolved a number of times in this class, and gonadal differentiation (although under genetic control) is responsive to experimental manipulations. In addition, as laboratory animals, amphibians are ideal; because females produce up to 10,000 eggs per clutch in some species; large sample sizes can be obtained. Besides, large number of individuals can be treated easily in experimental manipulations because larvae are typically aquatic and readily absorb hormones and other chemicals added to rearing water (Hayes, 1998).

Amphibian skin secretions may be used as a raw material for different types of research. It is hoped that such work will ultimately lead to a greater understanding of the functions of skin gland secretions in amphibians, particularly their anti-predator and antimicrobial roles. In addition, applied aspects of such work include the possibility of the discovery of totally novel molecular structures that may exhibit useful physiological, pharmacological or

antimicrobial properties. This is due to the fact that amphibians possess well-developed immune systems. Secretions from some species of toads (genus *Bufo*) are now known to be capable of both antibacterial and antifungal activity suggesting some form of antimicrobial defence system is present, probably on the skin surface (Clarke, 1997).

2.4. Chromosome features and importance in systematics

There are chromosomal differences between species, differences not only in the genes but also in the number, size, and shape of the chromosomes themselves (Orr, 1976). Chromosome studies can contribute useful information for phylogenic analysis. Chromosomes can be studied as a morphological manifestation of the genome in terms of their microscopically visible size, shape, number, and behaviour during cell divisions.

2.4.1. Chromosome features

For most of the cell cycle, eukaryotic chromosomes exist as loosely packed chromatin and cannot be distinguished in the nucleus. However, at the onset of mitosis or meiosis, they become visible due to chromatin condensation. Metaphase chromosomes form discrete structures, which stain densely when the nuclei are treated with appropriate dyes. Chromosome shape and length, centromeric position, telomeres, satellites, and nucleolar organizer regions are some of the morphological features of metaphase chromosomes. These morphological features allow individual chromosomes to be recognized and aberrations to be identified so that cytogenetic analyses are almost always based on examination of the chromosomes fixed during mitotic (or meiosis) metaphase (Twyman, 2003). Generally, metaphase chromosomes may differ from one another in size, shape, and relative and absolute length.

2.5.1.1. Chromosome number

The number (and morphology) of chromosome is characteristic of species, that is, virtually all individuals of a given species normally have the same chromosome number. The range in chromosome number between species is immense, as stated in Gupta (2004). In plants the chromosome number varies from 2n= 4 in *Haplopappus gracilis* (Compositae) to 2n =

> 1200 in some Pteridophytes. The known range of diploid chromosome number in amphibians is 14 to 104: 24 to 42 in gymnophiona, 22 to 64 in caudate and 14 to 104 in anurans (Fredga, 1977).

The chromosome complement of a species, in addition to the total number of chromosome, may be characterized by the total number of chromosome arms, which is referred to as fundamental number (FN). If the chromosome complement includes only the arms of the autosomal chromosomes, it is referred to as autosomal fundamental number (FNa) (Robbins and Baker, 1978).

2.5.1.2. Centromere

Centromere is an under-condensed region of the chromosome that interacts with the spindle fibers and ensures proper chromosome movement during nuclear division. When this process fails, the daughter cells will have abnormal chromosome number, which can lead to phenotypic abnormality or death (Griffiths, *et al*., 1999). The centromere (primary constriction) may be located on a particular part of the chromosome.

Corresponding to centromeric position, chromosomes may be classified as (i) metacentric: having centromere at the middle, (ii) submetacentric: having centromere slightly off center, such that one arm is shorter than the other, (iii) acrocentric: having centromere close to an end, but not at the end, and (iv) telocentric: having centromere located at the end (Twyman, 2003).

The relative position of the centromere is constant, which means that the ratio of lengths of the two arms (r = l/s, where r = arm ratio, l = long arm and s = short arm) is constant for each chromosome. This ratio is an important parameter for chromosome identification. As suggested by Levan *et al*. (1964), the position of centromere is named on the basis of arm ratio (r) that allows the classification of chromosomes into basic morphological types. The centromere may occupy median point (M), median region (m), submedian region (sm), subterminal region (St), terminal region (t) and terminal point (T), with the corresponding arm ratios of 1.0, 1.0-1.7, 1.7-3.0, 3.0-7.0, 7.0-∞ and ∞, respectively. On this basis,

chromosomes are thus categorized as M, m, sm, st, t, and T types, respectively.

2.5.1.3. Telomere

Telomeres are the region of DNA at the end of the linear eukaryotic chromosome that are required for the replication and stability of the chromosome. Telomeres of the chromosome are composed of specific tandem repeats of the DNA sequence that vary from species to species, but are generally GC-rich (Joeng *et al*., 2004). For example, tandem repeats of the DNA sequence TTAGGG are conserved in all species of vertebrates (McClean, 1997; Rocco, 2001).

According to Gupta (2004), if a chromosome breaks, the broken ends can fuse due to lack of telomeres. However, a chromosome cannot fuse at the telomeric end, which suggests that a telomere has a polarity that prevents other segments from joining with it.

2.5.1.4. Chromosome size

Chromosome is normally measured at mitotic metaphase and may be as short as 0.25μ (μ =. 001mm) in fungi and birds, or as long as 30 μ in some plants like *Trillium* (Gupta, 2004). Size difference of chromosomes between species can be over 100 fold and within a species some chromosomes are often 10 times as large as others (Weaver and Hedrick, 1997). According to Stebbins (1971), chromosome size, including the total DNA content of the nucleus which is designated as absolute chromosome size, may vary as much as 20 fold between genera of the same family having the same or similar basic chromosome numbers.

The chromosomes in the genome can also 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 provide a powerful means of characterizing genomes by means of their relative sizes and shapes, giving rise to the concept of karyotype, representing the appearance of chromosomes at mitotic metaphase. Differences in the karyotypes have aided many cyto-taxonomic decisions (Gupta, 2004).

The stage of mitosis in which chromosome is fixed is one of the factors that affect the absolute length of any one chromosome since condensation (shortening) of chromosomes progressively increases until it reaches maximal at metaphase.

Chromosome lengths may also be influenced to some extent by the type, concentration, and duration of agents used to arrest mitosis; type and concentration of salts used in hypotonic treatments and the type of fixative used (Dietrich, 1986; Ronne, 1989).

2.5.1.5. Secondary constriction and satellites

Besides the centromere, which produces a primary constriction in chromosome, secondary constrictions can also be observed in some chromosomes. Secondary constriction, if present in the distal region of an arm, would pinch off a small fragment called trabant or satellite. The satellite remains attached to the rest of chromosomal body by a thread of chromatin. Chromosomes having a satellite are marker chromosomes and are called SAT-chromosomes (Gupta, 2004).

Secondary constrictions, usually represented as nucleolar organizer regions (NORs), are sites around which nucleoli reorganize during telophase-interphase. These sites, during metaphase, contain under-condensed DNA identified as rDNA genes that are responsible for 80% of total RNA synthesis within eukaryotic cells (Saitoh and Laemmli, 1994).

Generally, different organisms have different number of nucleolar organizer chromosomes, and the position of NORs is highly specific in a chromosome set so that it can be used as useful markers (Gupta, 2004).

2.5.1.6. Chromosome banding

One method of identifying metaphase chromosomes is by their banding patterns, which are induced by specific treatment of chromosomes before staining. A band can be defined as certain part of a chromosome, which is clearly distinguished from its adjacent segments by being darker or brighter with one or more banding techniques. A variety of different kinds of bands have been produced in animal materials (Gupta, 2004).

Depending up on the nature of band produced, banding techniques can be categorized into two principal groups, these are (i) techniques that result in a restricted number of specific bands or structures such as C-banding which reveals constitutive hetrochromatin regions or silver staining of nucleolar organizer region and (ii) techniques that result in bands distributed along the length of the whole chromosome, such as G-, Q-, and R-bands.

G-banding produces patterns of light staining and dark-staining regions along the chromosome length. The banding pattern is similar for homologous chromosomes but it is different for non-homologous. R-banding results in a banding pattern that is the reverse of G-banding or Q-banding. Unfortunately, these bands are produced in chromosomes of amniotic vertebrates, not on chromosomes of lower vertebrates like amphibians. Rather, C-banding is more useful to study chromosomes of lower vertebrates.

Constitutive hetrochromatin of anuran chromosomes, as revealed by C-banding, presents a highly variable distribution, in qualitative and in quantitative respects and thus C-banding patterns have been used to differentiate very similar standard karyotypes (Hsu, 1975). For example, among the *Alsodes* species: *A. pehuenche*, *A. vanzolinii*, and *A. verrucosus*, have 26 chromosomes. C-banding karyotypes are useful in order to identify species and some chromosome groups, although they are not sufficient to resolve the phylogeny of *Alsodes* species group (Cuevas and Formas, 2003).

Silver nitrate stains nucleolus organizer regions at metaphase that have been active in the preceding interphase. NOR-banding is useful in the study of certain chromosome polymorphisms such as double satellites and it is also important for the identification and distribution of NORs in the chromosome complement. Silver staining shows that among *Alsodes* species, for example, most have only one pair of secondary constriction, except *A. kaweshkari* which has three pairs of secondary constriction (Cuevas and Formas, 2003).

2.5.2. Chromosome changes

Chromosome changes are numerical, if it involves a deviation from normal chromosome number, or structural, if it involves deviation from normal structure as the result of

breakage and rearrangement of chromosome segments.

2.5.2.1. Mechanisms of changes in chromosome number

Chromosome numerical changes could either be aneuploidy or euploidy. Aneuploidy results in gain or loss of chromosomes less then one complete set. Euploidy results in gain or loss of complete set(s) of chromosomes. Euploidy variants can arise from cell-cycle failure. For example, omission of DNA replication/unscheduled chromosome segregation (to halve the ploidy) or omission of chromosome segregation/ unscheduled DNA replication (to double the ploidy). Changes in ploidy can also arise at fertilization; triploidy in mammal for instance may result from fertilization of egg by two sperm (Twyman, 2003).

Aneuploidy, which is a gain or loss of one or a few chromosome (less than one complete set), arises in various ways. Non-conjunction (i.e. homologous chromosome fail to pairs during meiotic prophase), anaphase non-disjunction (i.e. chromosomal pair at meiosis or chromatid at mitosis fails to segregate), and lag of chromosomes at anaphase are the most common origins of aneuploidy.

The presence of extra, dispensable, chromosome chromosomes called B-chromosomes is another type of variation in chromosome number (Gibson, 1984). Their origin has remained a mystery, but it is proposed that they may be are remnants of chromosomal rearrangements (Camacho, *et al*., 2000). When present in a population, B-chromosome may not be necessarily found in all individuals of the population. Their numbers may vary between individuals as well as between different tissues of the same organism (Jones, 1995; Bougard and Jones, 1997). The presence of supernumerary chromosomes would change both chromosome number and the arm number, but if recognized as B-chromosome they are generally counted separately.

The number of chromosomes and / or the number of chromosome arms may be altered due to various types of chromosomal rearrangements. Results of some type of rearrangement may be less obvious while other may produce obvious chromosomal changes. For example, due to chromosomal rearrangements, genes may be duplicated or deleted, or DNA sequence

or linkage group may be changed, or the position of a gene may be changed. The level or pattern of gene expression may be altered as a result of chromosomal rearrangements that affect the position of a gene, a phenomenon known as position effect (Gibson, 1984).

Karyotypic fissioning refers to fissioning of the entire complement of chromosomes whereas tandem fusion is a fusion of two chromosomes in which the end of one chromosome is fused either to the end or to the centromere of other chromosome. Tandem fusion and Karyotypic fissioning may change both chromosome number and chromosome arm number. Karyotypic fissioning may lead to an odd number of chromosomes to be found in some individuals. In genus *Muntiakes*, *M. muntijak* has 2n = 6 in the female and 2n = 7 in the male (Marks, 1983). This is the case when one member of each of the two pairs of acrocentrics has fused to form a metacentric, but their respective homologs have remained separate. If one of the sex chromosomes is involved, it will result in chromosome number difference in male and female of a given species (Gropp and Winking, 1972).

The principal rearrangements of the chromosomes are inversions (peri- and paracentric) and translocations (Fredga, 1977). Robertsonian or whole arm translocations are extreme form of non-reciprocal translocation, where two acrocentric or telocentric chromosomes are fused at or near the centromere to generate a single chromosome (compound chromosome). It is also possible for a single metacentric or sub-metacentric chromosome to undergo fission (centric fission) of one centromere and generates two telocentric chromosomes (Twyman, 2003). These kinds of rearrangement change the chromosome number but not the arm number. If the position of the centromere is changed, as in the case of a pericentric inversion or unequal reciprocal translocation, the number of arms may be changed without changing the number of chromosome (Fredga, 1977).

Centric fissions and translocations are the main mechanisms that govern the chromosomal evolution of the frog genus *Alsodes* (Anura: Leptodactylidae). As mentioned in Cuevas and Formas (2003), within the genus *Alsodes*, three diploid number were detected; 2n = 22, 26, and 34. *Alsodes* species possessing karyotype with 26 chromosomes have a FN = 52. Exceptionally, *A. vanzolinii* was found to have a telocentric pair (pair 13) as a result of

translocation. In this case, the chromosome number remained constant (2n = 26), whereas the FN changed from 52 to 50. Likewise, *A. barrioi* (2n = 34, FN = 52) were presented an unusual karyotype with eight telocentric chromosomes. In order to explain the origin of these karyotype, Cuevas and Formas (2003) proposed the phenomena of centric fission took place in a hypothetical primitive karyotype with 26 chromosomes. In this case, the FN remained constant (52) but the chromosome number changes from 26 to 34.

2.5.2.2. Mechanisms of changes in chromosome morphology

According to White (1978), one mechanism that has been proposed as important in rapid speciation is change in chromosome structure. Chromosomes may change in structure without changing in either the chromosome number or the arm number. A pericentric inversion will change the order of genes on a chromosome with or without changing the shape or size of the chromosomes (Bogart, 1974). On the other hand, translocation between unlike chromosomes result in a change in shape of the chromosomes, but in both cases (pericentric inversion and translocation) without changing the total number of chromosomes or chromosome arms. In the frog genus *Leptodactylus*, for example, *L. andreae* has the same number of chromosomes as *L. hylaedactylus* but has only half as many pairs of t-chromosomes. This difference is the result of pericentric inversions involving at least three pairs of chromosomes (Bogart, 1974).

Length of chromosomes may change due to deletion or duplication. Deficiency or deletion is a phenomenon in which a chromosomal segment or gene is missing so that it reduce the length of the chromosome. Duplication, on the other-hand, is a situation in which a chromosomal segment or gene is represented more than once in haploid genome and makes the chromosome longer. Translocation is another cause for which chromosomes may increase or decrease in size as a result of transfer of chromosomal segment from one chromosome to another non-homologous chromosome (Twyman, 2003).

Generally, data on chromosome morphology, when applied with other criteria, can play an important role in the study of taxonomy and evolution of Anurans. As mentioned in Cuevas and Formas (2003), different karyological mechanisms (fusions, fissions and translocations)

have been used to explain the karyological evolution in frogs.

2.5.3. Application of karyotype data in amphibian taxonomy

Different techniques of chromosome staining such as banding technique allow the identification of homologous chromosomes and the chromosomes to be arranged as a karyotype. The term karyotype is given to a size-organized set of photographs of chromosomes. Karyotype of a given individual can be constructed from chromosomes arranged by either cutting out chromosome photographic prints and pasting the homologous pairs on white board or by using computer programs developed for this purpose. According to Gupta (2004), the karyotype of different groups of organisms may be compared and similarities in karyotypes are presumed to represent evolutionary relationships.

Most animals have karyotypes that are characteristic for the species to which they belong (Fredga, 1977). In some cases, however, a given karyotype may characterize several species or genera or even families. For example, among the genera of a Ranidae family which have basic karyotype 2n = 26, FN = 52; five species of the genus *Ptychadena* are karyotipically similar (2n = 26, FN = 48), whereas genus *Phrynobatrachus* is karyotipically non-conserved. Three different chromosome number (2n= 16, 18, and 20) had been found among the six *Phrynobatrachus* species and each of the species had a distinctive karyotype (Bogart and Tandy, 1981).

In contrast to the above, a given species may have different karyotypes. According to Bogart (1968), the diploid chromosome number of *Bufo regularis* was consistently reported to be 22 chromosomes worldwide. However, African *B. regularis* has a diploid complement of only 20 chromosomes. It is conceivable that there was an early dichotomy in Africa such that a change in chromosome number occurred at one time. This would suggest that all of the toads possessing 20 chromosomes might be derived from a common ancestor.

Great cytological disparity may occur in some groups in spite of overwhelming morphological similarity and hybridization potential between the groups. The most

interesting example of this kind is found in the mammalian genus *Muntiacus*: *M. reeves*; both sexes have 2n = 46, whereas *M. muntijak* has 2n = 6 in the females and 2n = 7 in the males. This reveals that the evolution of chromosomes in this group has proceeded much more rapidly than the evolution of either the morphology of the organisms or their genes. Tachytely is a term applied to chromosome evolution that denotes exceedingly rapid within a group, while bradytely can be defined broadly as karyotypic conservatism within a taxonomic group relative to the degree of diversity displayed at other levels of analysis. Such exceedingly slow evolutionary process is known from anurans (Marks, 1983).

The karyotype information from frog family Ranidae has been used to exemplify chromosomal conservation. Even if variation in chromosome number and morphology were encountered with the analysis of chromosomes obtained from 52 species of African ranid frogs, the karyotypes provide evidence for two major chromosome groupings derived from an early dichotomy which resulted in two ancestral karyotypes having 2n = 26 and 2n = 24 chromosomes. Further chromosomal diversification leads to variety of karyotypes with diploid numbers ranging from 14 to 54. The genera *Anhydrophryne*, *Chromantis*, *Hylarana*, *Petropedete*, *Rana*, and *Tomopterna* have species, which possess generalized karyotypes with five pairs of larger chromosomes and a sharp distinction between the large and small chromosome pairs. On the other hand, variation of chromosome number in *Leptopelis*, C*ardioglossa*, and *Phrynobatrachus* are useful to outline species groupings within these genera (Bogart and Tandy, 1981).

According to Bogart (1974), the 24 and 26 karyotypes were derived from an original 22chromosome karyotype by means of centric dissociation and pericentric inversion if karyotypic evolution is parallel to evolutionary trend.

Chromosome numbers are known for only a fraction of the approximately 2500 species and 245 genera of anurans. Although many more genera and some additional families need to be studied, the general trend of higher number of chromosomes in primitive families and lower numbers in advanced families seems to exist in anurans (Duellman, 1967). In the genus *Phrynobatrachus*, which have 2n = 16, 18 and 20 chromosome grouping, the

evolutionary trend seems to be chromosome number reduction which has occurred at least twice. Even more striking example of chromosome number reductions were found in *Arthroleptis* (2n = 14) and it has the lowest chromosome number of any anuran (Bogart and Tandy, 1981). However, it is highly doubtful in some cases that higher chromosome number is indicative of primitiveness. For instance, in the family Hylidae, the *Hyla microcephala* (2n = 30) certainly is not the primitive group of *hylids.* In general, when applied with other criteria, enough data on the karyotypes and chromosome number might be useful in the study of phylogenetic relationships of anuran amphibians (Duellman, 1967).

It is possible to suggest whether the condition of a given karyotype is primitive or advanced. A-karyotype (asymmetrical) of caudate amphibians is characterized by high chromosome number with two types (small and large) and asymmetric in centromere location (i.e. larger metacentric and small telocentric of chromosomes). This has been considered to represent a primitive condition and it can be further divided into binomial (Ab) type with micro-chromosomes (e.g. *Andrias davidians* which has 2n = 60) and unimodal (Au) type without micro-chromosomes. Centromere fusion and pericentric inversions on either Ab or Au type lead to S (single-type symmetric) karyotype, which is considered to be advanced and characterized by fewer chromosome numbers. In these processes, total number of chromosome gradually decreases (i.e. elimination of micro-chromosomes) while the number of two-armed chromosome increases. The general process is Ab → Au → S through loss of centromere (Zhu, *et al.*, 2002).

Various experiments convincingly showed that both XY/XX and ZW/ZZ sex determining mechanisms co-exist in the primitive as well as in highly evolved group of urodela and anuran amphibians (Schimid, *et al*., 1990). Even though reported hetromorphic sex chromosome cases are rare among amphibians (Zhu, *et al*., 2002), the improvement of cytogenetic methods for the analysis of chromosome structures in mitosis and meiosis made it possible over the last years to definitively demonstrate that heteromorphic sex chromosomes have been developed in the evolution of amphibian genome. The first certified heteromorphic sex chromosomes in anura, which possesses female ZW/ male ZZ system of sex determination, were discovered in South African bullfrog *Pyxicephalus*

adspersus. The 'W' is considerably smaller than 'Z' and its short arm is completely heterochromatic. In addition, highly heteromorphic sex chromosomes of XY / XX type were found in marsupial *Gasrotheca riobambae*. The 'Y' is the largest element in the karyotype and almost completely heterochromatic (Schimid, *et al*., 1990).

3. OBJECTIVE OF THE STUDY

3.1. General objective

The objective of the study is to investigate the chromosome of some species of Ethiopian anuran amphibians.

3.2. Specific objectives

The specific objectives of this study are:

- To document the chromosome number of amphibian species studied.
- To describe chromosome morphology and characterize the karyotype of amphibian species studied.
- To compare the karyotypes of studied amphibian species with those of related species reported by other workers to see evolutionary changes in the karyotypes.

4. MATERIALS AND METHODS

4.1. Specimens collection

4.1.1. Sites of collection

Specimens were collected from six localities in Ethiopia (Fig.1). The geographic location of each locality and the number of specimens collected are presented in Table 3. These locations were selected based on their richness in amphibian diversity and suitability of conditions such as availability of transportation, occurrences of surface water and swamps.

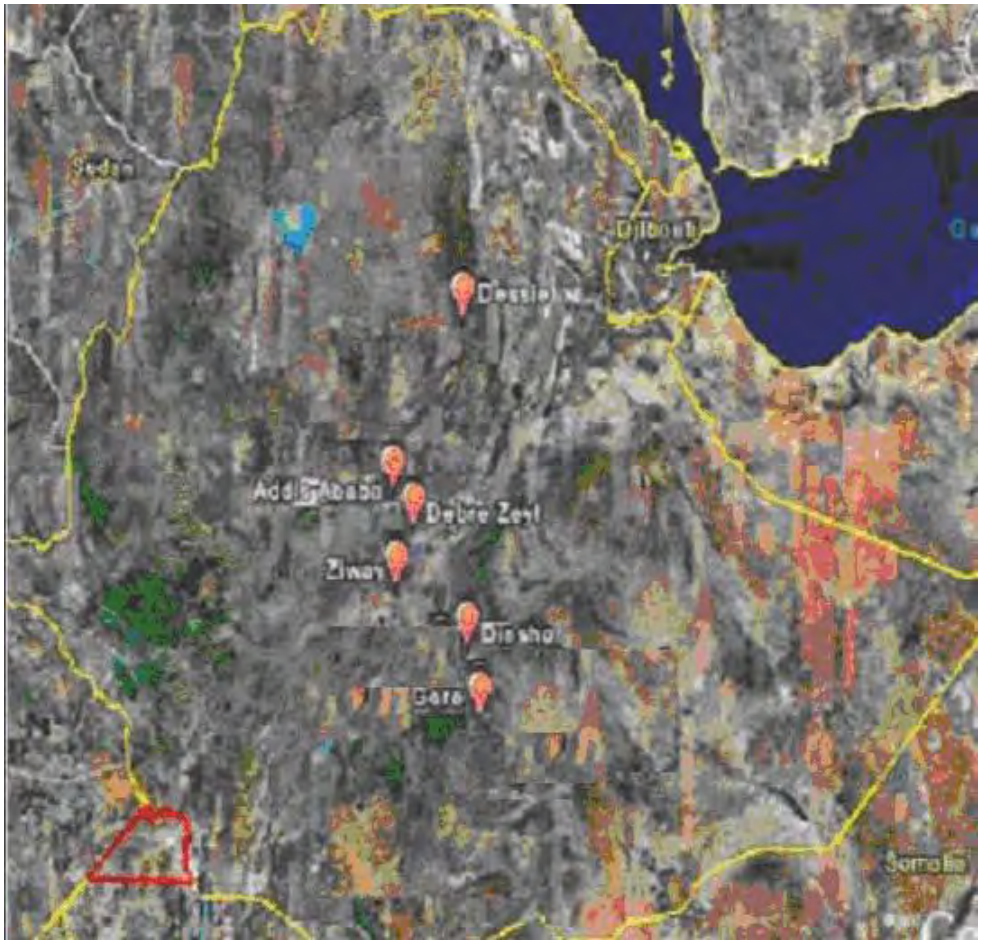

Fig. 1. Map Ethiopia of showing the sites of specimen collection.

Table 3. Species of amphibians, the geographic locations and number of amphibians captured.

Species	Locality	Geographic location		No. of Amp. Captured		
		Position	**Altitude**	**M.**	**F.**	**Total**
Rana angolensis	Addis Ababa	$9^0$2'N $38^0$54'E	2400-2700	**2**	**3**	**5**
Xenopus clivii	Addis Ababa	$9^0$2'N $38^0$54'E	2400-2700	**6**	**3**	**9**
	Dinsho	$7^0$6'N $39^0$46'E	3100	**1**	**2**	**3**
Hyperoliidae spp. *	Addis Ababa	$9^0$2'N $38^0$54'E	2400-2700	-	-	1
Ptychadena cooperi	Addis Ababa	$9^0$2'N $38^0$54'E	2400-2700	-	**2**	**2**
Ptychadena anchietae	Goro	$6^0$5'N $40^0$02'E	2690	**1**	**1**	**2**
Ptychadena neumanni	Dessie	$11^0$04'N $39^0$43'E	2000	**3**	-	**3**
	Ziway	$7^0$55'N $38^0$43'E	1650	**2**	**2**	**4**
Bufo regularis	Debre Zeit	$8^0$44'N $38^0$59'E	2000	**1**	**1**	**2**
	Goro	$6^0$5'N $40^0$02'E	2690	**2**	-	**2**
Bufo garmani	Dessie	$11^0$04'N $39^0$43'E	2500	**2**	-	**2**
	Debre Zeit	$8^0$44'N $38^0$59'E	2000	**2**	**3**	**5**
			Total	**25**	**17**	**40**

* Sex unidentified because the specimen has not fully completed the tadpole stage.

4.1.2. Method of capturing specimens

For successful collection of the amphibians, a long handled scooping net was used for those species that inhabit aquatic environments. Besides, hand collection method was also used where the depth of the water was low enough to be reached, and in terrestrial amphibians. The captured specimens were transferred to plastic bottles containing water for temporary handling. The photographs of some captured representative amphibians are shown in Fig. 2.

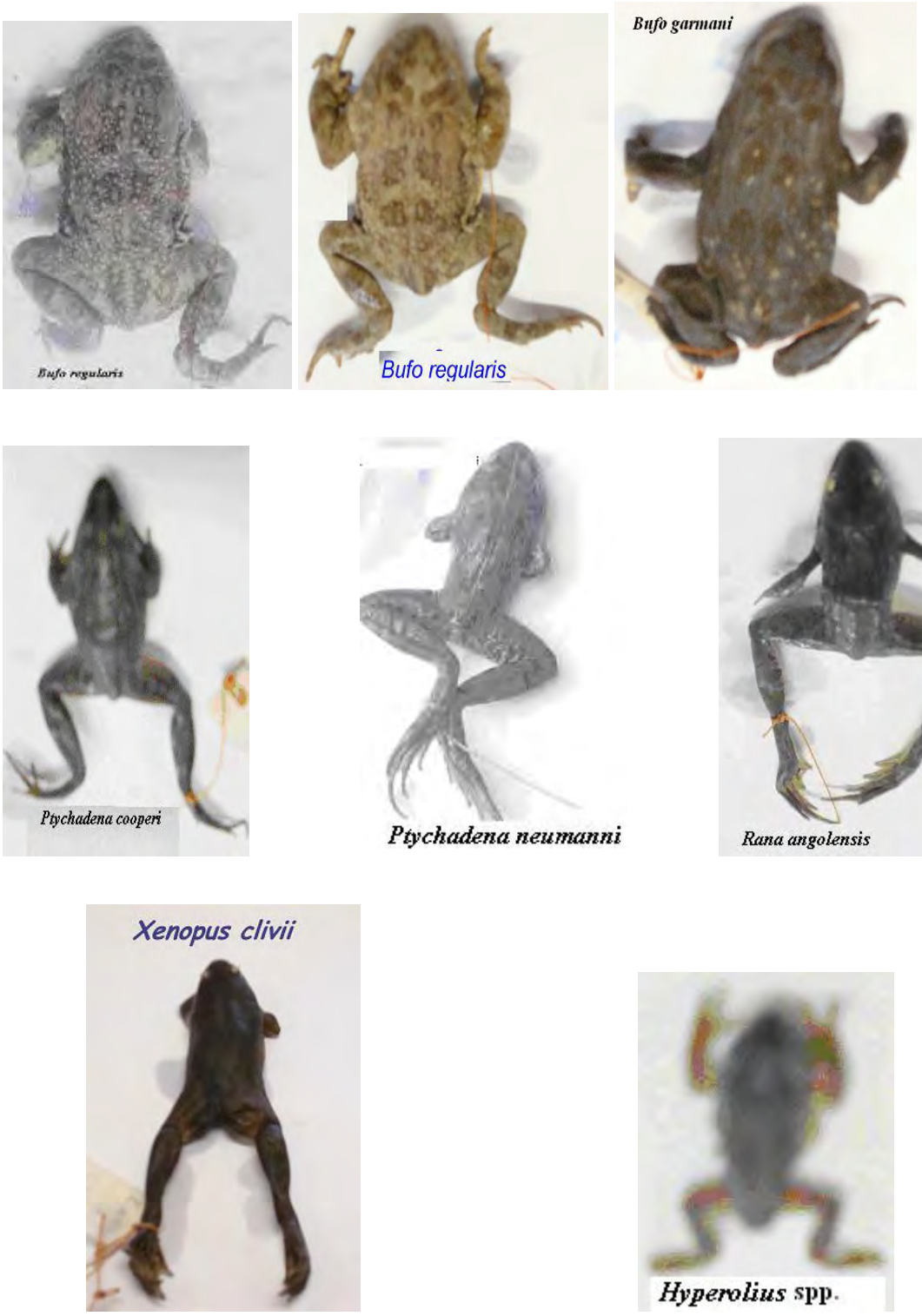

Fig. 2. Specimens captured from different localities in Ethiopia.

4.1.3. Preservation of specimens

Collected amphibians were preserved in 70 % ethanol and the containers were sealed to avoid evaporation (Etheridge, 1996). The preservation was done after the amphibians were killed by over-anaesthesia and dissected to remove some organs (intestine, kidney, stomach) for chromosome preparation. The specimens were properly labeled, preserved and kept in the Genetic Laboratory, at the Science Faculty of Addis Ababa University, for later deposition in the Natural History Museum, Department of Biology, Science Faculty of Addis Ababa University. Identification of preserved specimens were made by amphibian taxonomist.

4.2. Somatic chromosome study

4.2.1. Metaphase chromosome slide preparation

Somatic c-metaphase chromosomes were prepared from intestinal epithelium cells using a technique modified from Sessions, *et al*., (1996). Individual animals were injected 0.1% colchicines at approximately 1ml/g body weight and left in a moistened plastic jar. After 10 - 17 hrs, the animals were killed by over-anaesthesia and dissected using fine pointed scissors. The stomach and intestine were removed, and opened lengthwise. Portion of these organs were homogenized in hypotonic solution of 0.075M KCl and kept in the solution for a total of 30 minutes. The cell suspension from the homogenized tissue was centrifuged at 1000rpm for about 5 minutes. The supernatant was removed using Pasteur pipette and the pellet was suspend in freshly prepared fixative (3:1, methanol: acetic acid v/v) for at least 30 minutes, and then centrifuged. After a total of three rounds of suspensions in fixative followed by centrifugation, the pellet was re-suspended in a small volume of the fixative. Several slides were prepared from each specimen by splashing a few drops of the cell suspension on a glass slide from a height of about one meter. The slides were air-dried at room temperature and stored until needed for staining.

In the field where centrifugation facilities were not available, a slight modification of the above method was used. The stomach and intestine were placed in the hypotonic solution for about 30 minutes and then transferred to the fixative and brought to the lab. The tissues

were homogenized in the fixative, and the centrifugation processes of the above were used.

4.2.2. Giemsa staining

Air-dried slides were stained with Giemsa stain in phosphate buffer (pH = 6.8). After 30 minutes or more, the slides were rinsed in distilled water, air-dried at room temperature and mounted with DPX under a 24x50mm cover slip.

4.2.3. Karyotype Analysis

Chromosomes were described and characterized using photomicrographs and direct observation under the microscope. For this purpose, photomicrographs of metaphase plates with good chromosome spreads were taken using a camera-fitted microscope with a magnification of x1000 (i.e., x10 eyepiece and x100 objective). Chromosome size, arm length, and arm ratios of the chromosomes were computed using a micromeasure computer program. The fundamental number of each karyotype was expressed as total number of chromosome arms. The chromosomes were then grouped into pairs (karyotyped) according to their size and centromere position. The lengths of each chromosome were expressed as a percentage of the total haploid complement length. The resultant percentage then multiplied by ten to avoid decimals and produce units of normalized length which could be used for chromosome nomenclature.

Chromosome classification as m, sm, st, and t was determined according to Levan *et al*. (1964) based on arm ration, r, (r = L/S; L = long arm, S = short arm) with some modifications. Median point and median region were classified as metacentric (m), when r = 1.00 - 1.69; submedian as submetacentric (sm), when r = 1.70 - 2.99; subterminal as subteocentric (st) or acrocentric, when r = 3.00 - 6.99; and terminal region and terminal point as telocentric (t) chromosomes, when r = 7.00 - ∞. Chromosomes were also classified as being small, medium, or large according to their normalized length as suggested by Bogart (1974). Chromosomes, which have a value of greater or equal to 100 units (i.e., 10% or more of the total haploid complement) are grouped as large, whereas those having a value below or equal to 80 units (i.e., 8% or less of the total haploid complement) are classified as small chromosomes. Chromosomes whose length falls between 80 and 100

units are considered to be intermediate in size.

The actual sizes of the chromosomes in μm were determined by using a stage micrometer, in which 1 mm is divided into 100 parts (i.e. each part being 0.01mm). The stage micrometer was photographed and printed as the same magnification as that of the chromosomes pictures. The enlarged pictures of the chromosomes were then measured with a cm ruler and the measurements were converted to μm.

5. RESULT

A total of eight species, seven identified and one unidentified, toads and frogs: *Rana angolensis, Xenopus clivii, Hyperoliidae* spp., *Ptychadena anchietae, Ptychadena neumanni, Ptychadena cooperi, Bufo regularis,* and *Bufo garmani;* collected from different localities of Ethiopia (Fig. 1) were studied and the results are presented in Table 4. The chromosome counts were based on maximum counts from several intact cells.

Karyotype analysis, including chromosome number, arm ratio, and total length and normalized length of each chromosome are presented in Appendices 4 - 13. The number of amphibian specimens studied with their respective number of metaphase cells analyzed from each locality are shown in Appendix 2 and karyotype analysis of haploid chromosome complement also presented (Appendix 3).

5.1. Karyotype description of *Rana angolensis.*

Five specimens of *Rana angolensis* from Addis Ababa coded as A-ST_1, A-ST_2, A-ST_3, A-AF_1, and A-EK9 were studied. Karyotype analysis was made from three specimens (A-ST_1, A-AF_1, and A-EK_9) and descriptions of the chromosomes are based on the analysis of 13 metaphase cells (Appendix 2). Representatives of mitotic metaphase chromosome spreads (Fig.3) and karyotype (Fig.4) are presented.

All the cells from above specimens have 2n = 26 and FN = 50. The chromosome complement consists of 6 metacentrics, 16 submetacentrics, 2 subtelocentrics, and 2 telocentric chromosomes. When chromosomes are arranged into pairs of decreasing length, a distinct gap in relative length is evident between pairs 5 and 6, and the chromosomes are divided into two groups: pairs 1-5 are large (>100 units) and pairs 6-13 are small (< 80 units) chromosomes. Besides, one pair of the small subtelocentrics chromosome (pair 6) from male specimen show size polymorphism and they probably constitute a pair of sex-chromosomes (Appendix 3). The karyotype formula is 6 m + 16 sm + 2 st + 2 t (Table 4).

Karyotype from Entoto Kidanemhiret (A-EK$_9$) specimens is not presented due to lack of cells with good chromosome spreads. However, visual analysis under microscope and photomicrographs has shown that they have similar karyotype as that of the rest of the specimens.

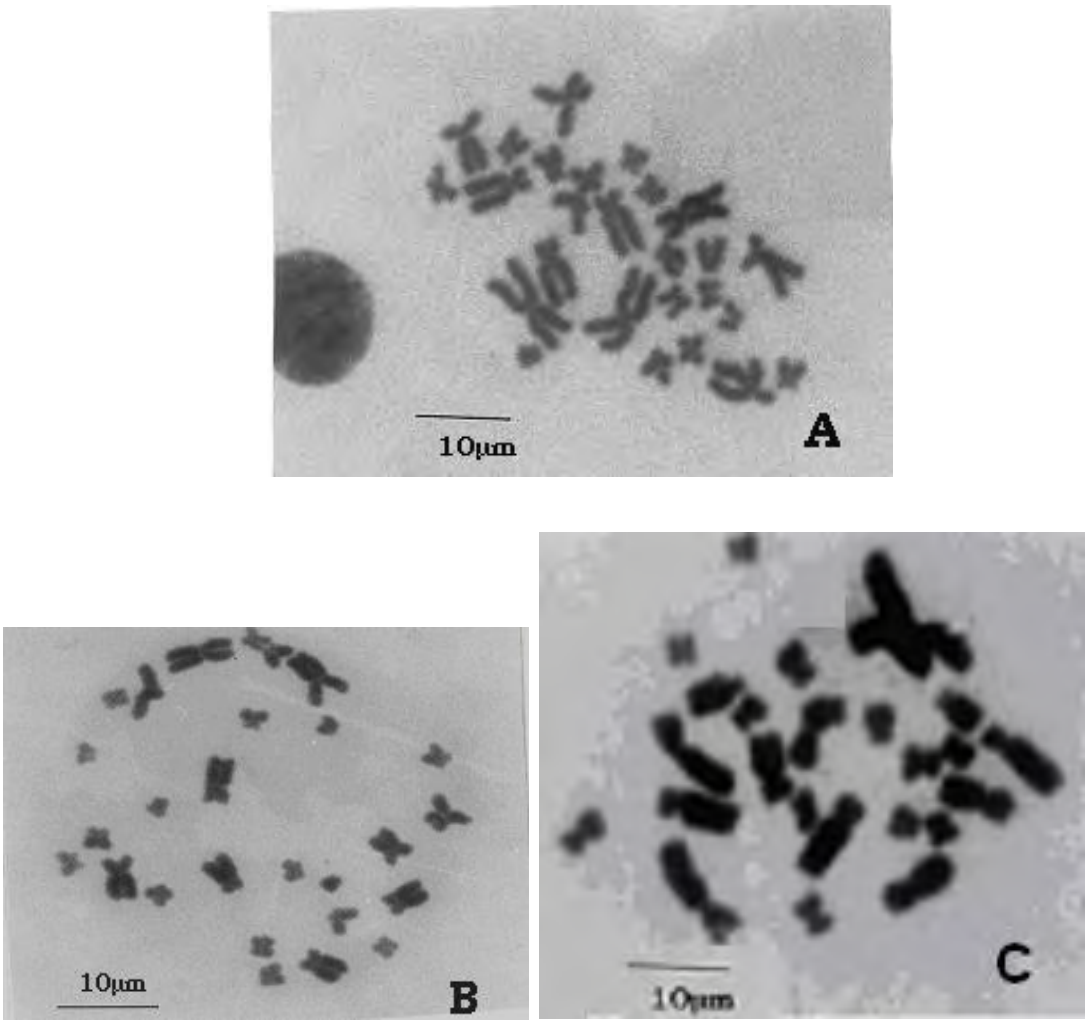

Fig. 3. Somatic metaphase chromosome spread of *Rana angolensis* specimens from Addis Ababa; (A) A-AF$_1$ (Female), (B) A-ST$_1$ (Male), and (C) A-EK$_9$ (Female).

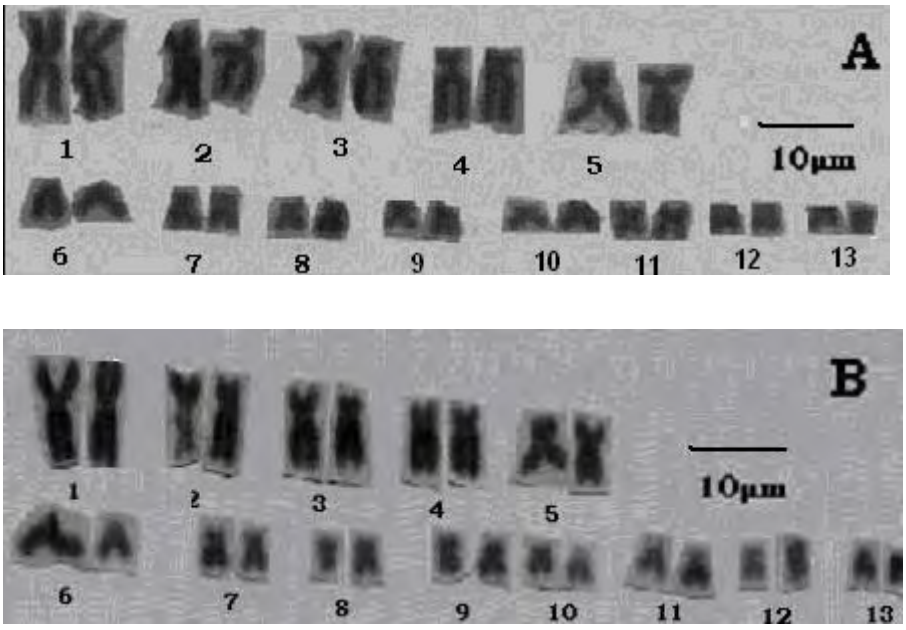

Fig. 4. Karyotypes of *Rana angolensis* specimens from Addis Ababa; (A) A-AF$_1$ (Female) and (B) A-ST$_1$ (Male).

5.2. Karyotype description of *Xenopus clivii.*

Somatic metaphase chromosome analysis has been made from one specimen captured from Addis Ababa (A-EK$_3$) and description of the chromosomes is based on the analysis of five metaphase cells (Appendix 2). Mitotic metaphase chromosome spread and karyotype are shown in Fig. 5. All the cells have 2n = 36 chromosomes, all bi-armed, so that the FN = 72. When the chromosomes are arranged into pairs of decreasing orders, all the chromosome pairs are small (< 80 units). Chromosome complement consists of seven pairs (pair 1, 2, 3, 6, 15, 16, and 17) of metacentric, three pairs (pair 4, 5, and 11) of submetacentric, and eight pairs (pair 7, 8, 9, 10, 12, 13, 14, and 18) of subtelocentric chromosomes. The karyotypic formula is 14 m + 6 sm + 16 st (Table 4).

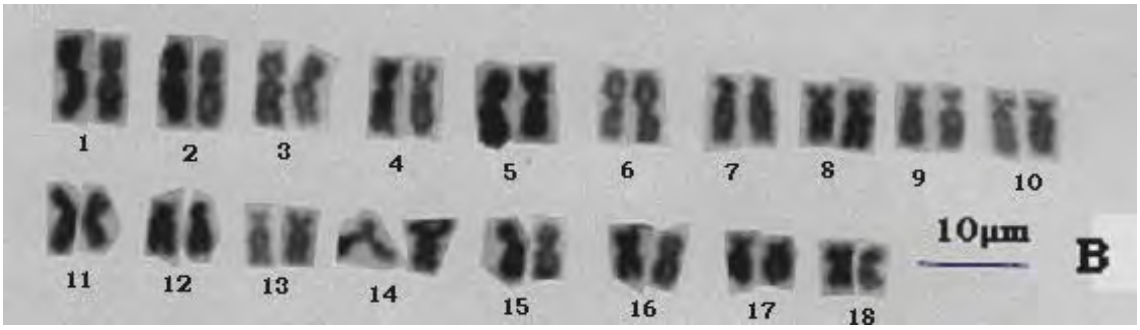

Fig. 5. (A) Somatic metaphase chromosome spread and (B) Karyotype of male *Xenopus clivii*

5.3. Karyotype description of *Hyperolius* spp.

Somatic chromosome analysis has been made only from one specimen of this species collected from Addis Ababa, Entoto Kidane Miheret (A-EK_{10}). Metaphase chromosome spread and karyotype are presented (Fig. 6). All the cells have 2n = 24 and FN = 48.

All the chromosomes are bi-armed which can be grouped into eight pairs of metacentric (pair 1, 6 - 12), two pairs of submetacentric chromosomes (pair 2, and 5), and two pairs of subtelocentric (pair 3 and 4) (Appendix 3). When the chromosomes are arranged into pairs, the karyotype is comprised of four pairs of large (>100 units), one pair of intermediate (between 80 and 100 units), and seven pairs small chromosomes (< 80 units) (Appendix 3).

The karyotypic formula is 16 m + 4sm + 4 st (Table 4).

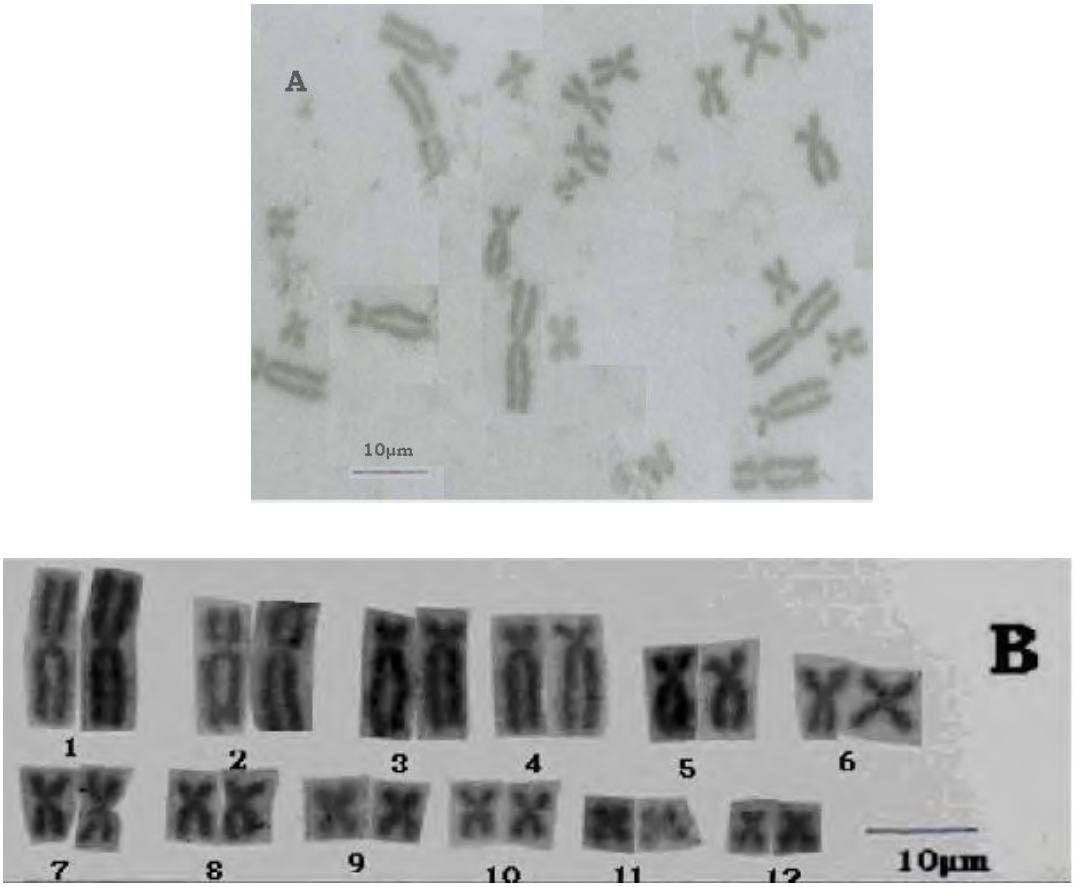

Fig. 6. (A**)** Somatic metaphase chromosome spread and (B) Karyotype of *Hyperolius* specimens from Addis Ababa.

5.4. Karyotype description of *Ptychadena neumanni*

Description of the chromosomes of this species is based on specimens captured from Ziway (Z - L_1, Z - L_2, Z - L_3, and Z - L_4) and Dessie-Tenta (D - T_1, D - T_4, and D - T_5). Representative somatic metaphase chromosome spreads (Fig. 7) and karyotype (Fig. 8) is presented. The chromosome complement of all the specimens consists of 24 chromosomes (2n = 24), all with two arms (FN = 48). Chromosomes are divided into three groups, large chromosomes (pairs 14), intermediate (pair 5 and 6), and small chromosomes (pair 7-12).

All the chromosomes are metacentric, except one pair (pair 3), which is submetacentric (Appendix 3). The karyotypic formula is 22m + 2 sm (Table 4).

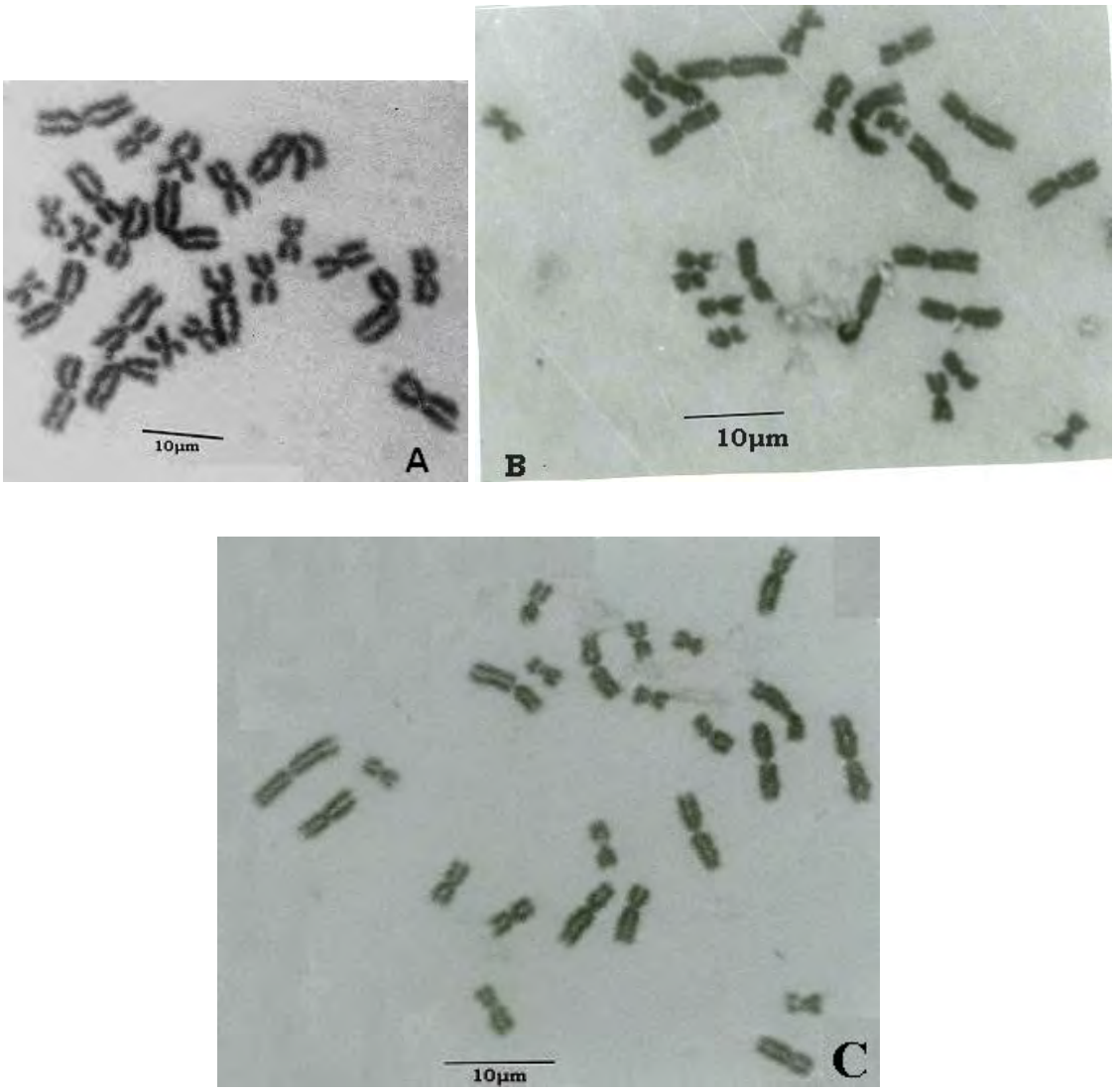

Fig. 7. Somatic metaphase chromosome spread of *Ptychadena neumanni*; (A) Male specimen from Ziway (Z - L_1), (B) Female specimen from Ziway (Z - L_2), and (C) Male specimen from Dessie-Tenta (D - T_5).

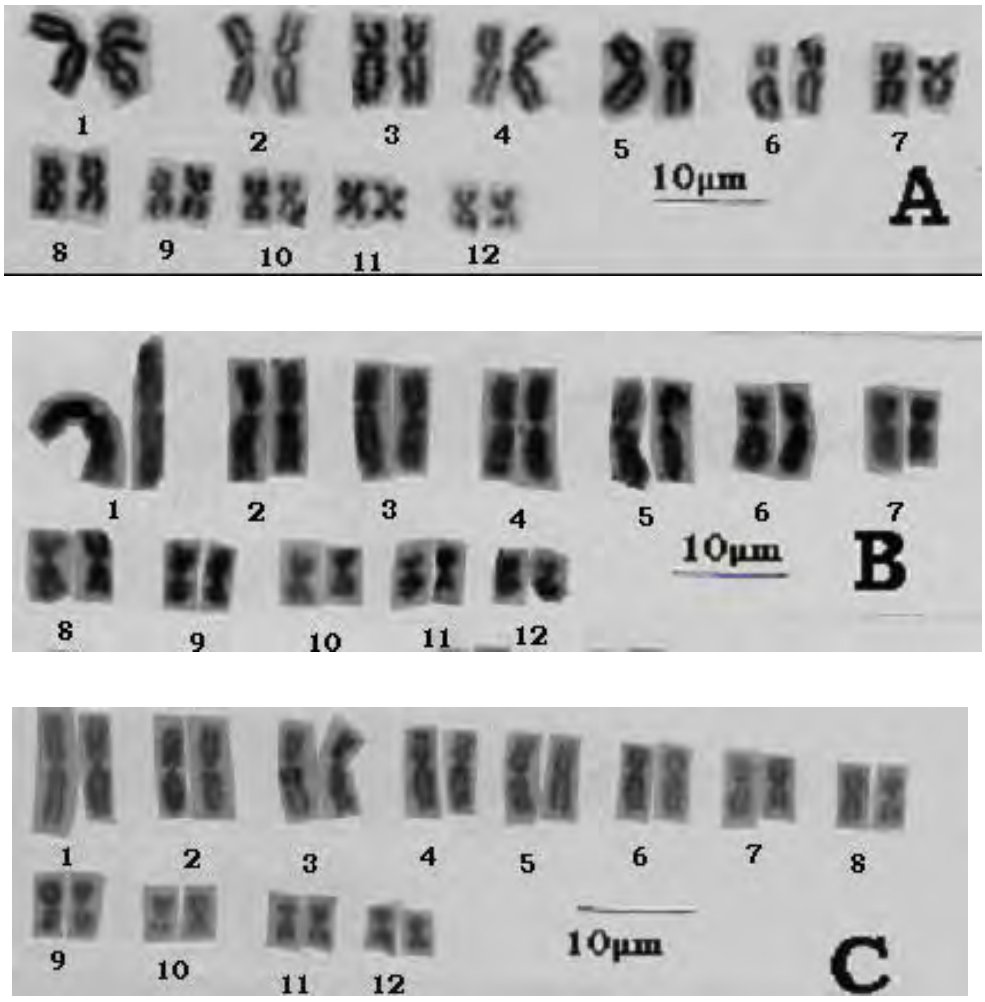

Fig. 8. Karyotypes of *Ptychadena neumanni* specimens; (A) Male from Ziway (Z - L_1), (B) Female from Ziway (Z - L_2), and (C) Male specimen from Dessie (D - T_5).

5.5. Karyotype description of *Ptychadena cooperi*

Somatic metaphase chromosome analysis of *Ptychadena cooperi* has been made from two specimens collected from Addis Ababa (A-K_1 and A-K_2) and chromosome description is based on the analysis of five metaphase figures. In all the metaphase plates, the chromosome number observed was 2n = 24 and FN = 48. A representative metaphase spread and karyotype of this species are presented in Fig. 9, which shows that *Ptychadena cooperi* has two type of chromosomes based on centromeric position; m and sm; that

comprise 11 pairs (1, 2, 4-12) and one pairs (pair 3), respectively. Pairs 1-4 are large (>100 units), pairs 5 and 6 are intermediate (between 80 and 100 units), and pairs 7-12 are small chromosomes (< 80 units) (Appendix 3). The karyotypic formula is 22 m + 2 sm (Table 4).

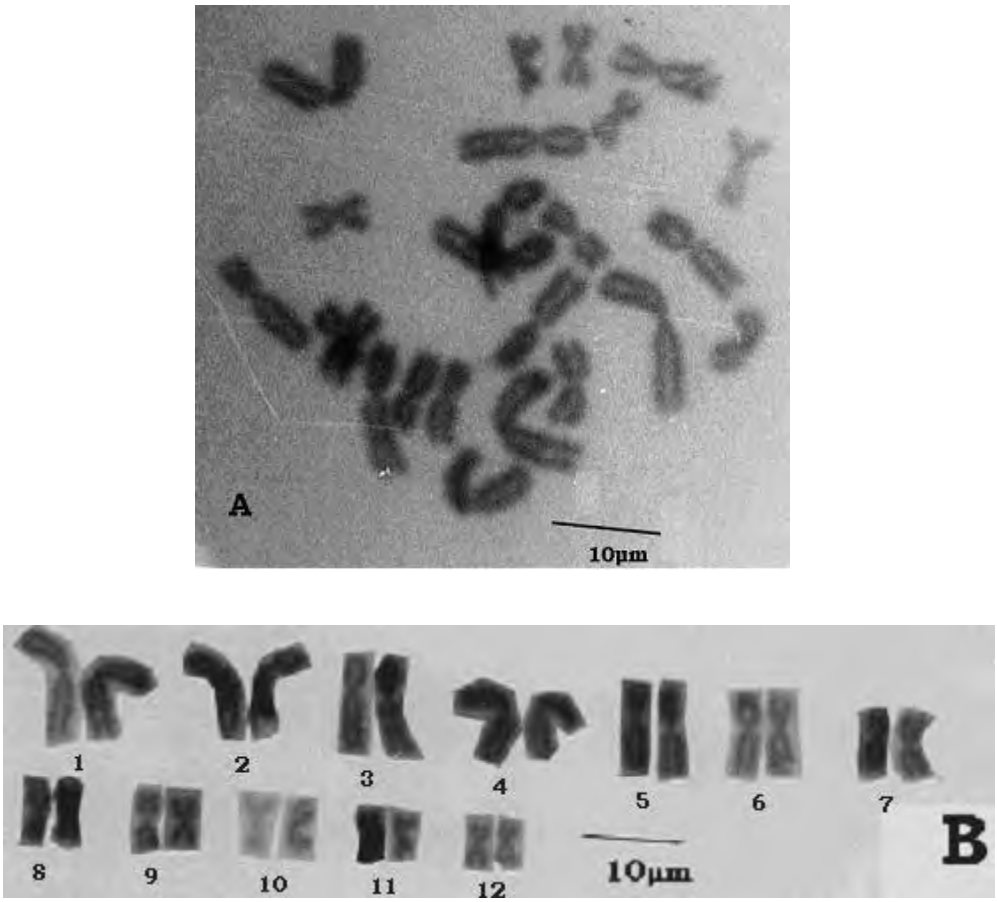

Fig. 9. (A) Somatic metaphase chromosome spread and (B) Karyotypes of female *Ptychadena cooperi*.

5.6. Karyotype description of *Ptychadena anchietae*

Specimens of this species were captured from Goro (G-R_1 and G-R_2). Somatic metaphase chromosome spread is presented if Fig. 10. The chromosome number of all metaphase cell have 2n = 24. Karyotype and chromosome measurements from these specimens are not presented due to most chromosomes of the cells that have relatively good chromosome spreads lack distinctive morphological features. However, chromosome size variation

within a spread is more or less similar as that of other species of the genus studied in this paper.

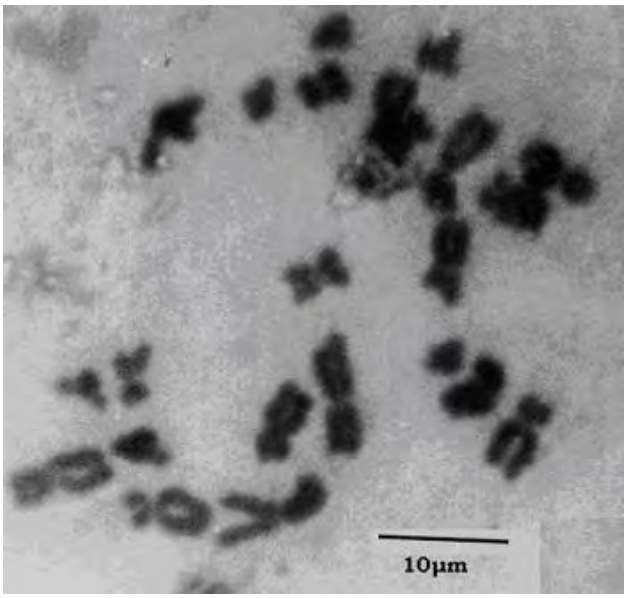

Fig. 10. Somatic metaphase chromosome spread karyotype of *Ptychadena anchietae* specimen (female) from Goro (G-R_1).

5.7. Karyotype description of *Bufo garmani*

A 2n = 20 and FN = 40 chromosomes were observed for all specimens of this species captured from Debre Zeit (DZ-C_8 and DZ-C_{11}). Representative somatic metaphase chromosome spreads (Fig. 11) and karyotypes (Fig. 12) are presented. When the chromosomes are arranged into pairs; a distinct gap in relative length is evident between pairs 6 and 7. Chromosomes are divided into three groups, large (pair 1-4), intermediate (pair 5 and 6), and small chromosomes (pair 7-10). Pairs 1, 2, 3, 5-9 are metacentric and pairs 4 and 10 are submetacentric chromosomes (Appendix 3). The karyotypic formula is 16 m + 4 sm (Table 4).

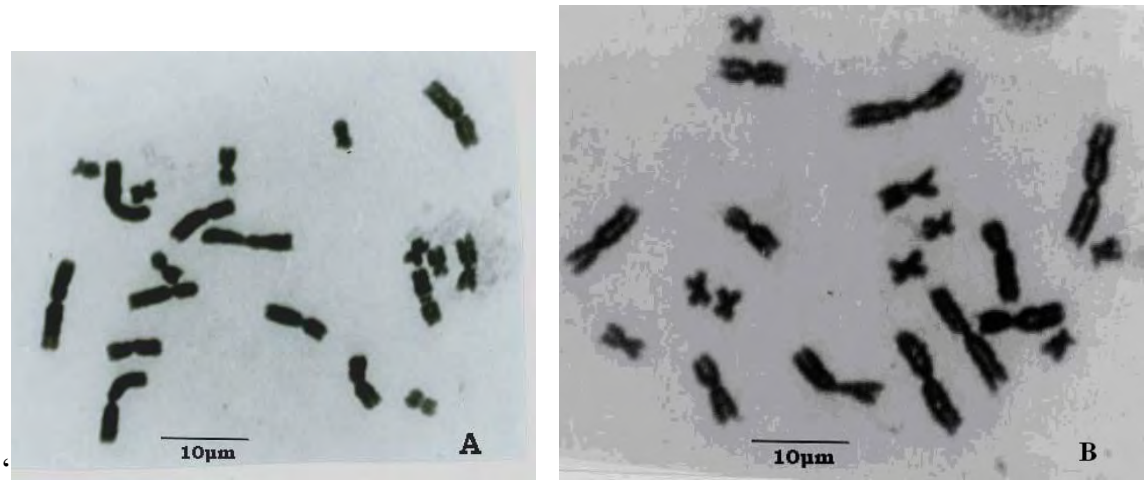

Fig. 11. Somatic metaphase chromosome spread of *Bufo garmani* specimens from Debre Zeit; (A) Female (DZ-C_8), and (B) Male (DZ-C_{11}).

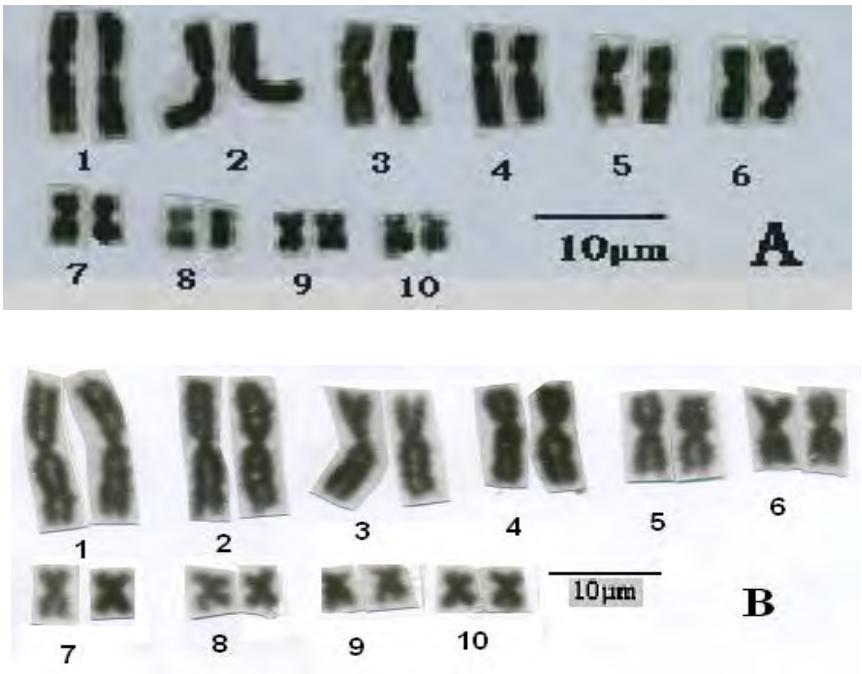

Fig. 12. Karyotypes of *Bufo garmani* specimens from Debre Zeit; (A) Female (DZ-C_8) and (B) Male (DZ-C_{11}).

5.8. Karyotype description of *Bufo regularis*

The description of the karyotype of *Bufo regularis* is based on the analysis of four well spread mitotic plates prepared from Debre Zeit (DZ-C_2) specimens. The chromosome complement of this species (Fig. 13) consists of 20 chromosomes, all with two arms (FN = 40). A distinct gap in relative length is evident between pairs 6 and 7. Chromosomes are divided into large (pair 1-3), intermediate (pair 4 - 6), and small (pair 7-10) and it also grouped into metacentric (pair 1, 2, 3, 5 - 9), and submetacentric (pair 4) chromosomes (Appendix 3). One pair of the metacentrics (pair 7) shows size polymorphism and they probably constitute a pair of sex chromosomes. The karyotypic formula is 16m + 4 sm (Table 4).

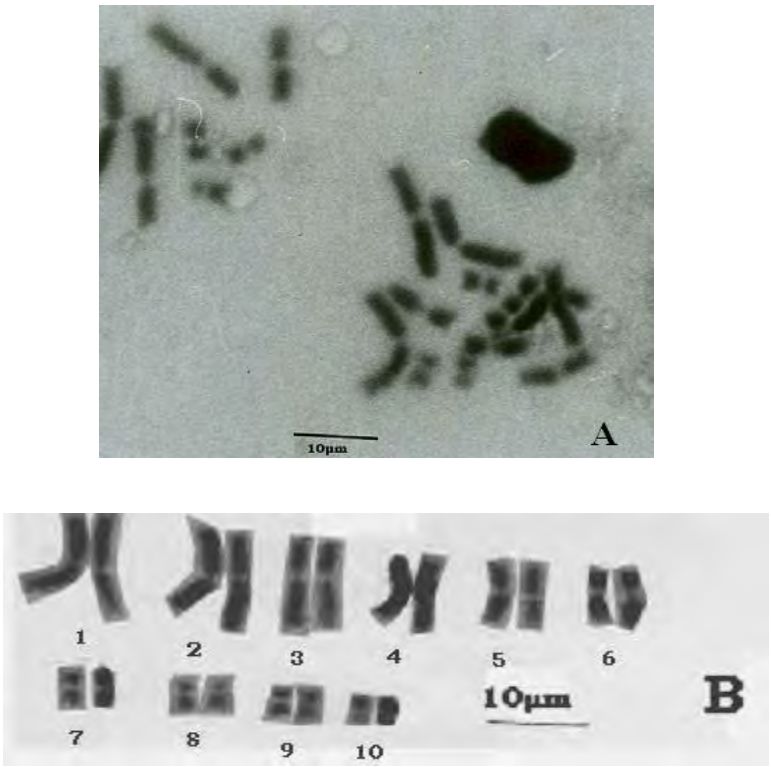

Fig. 13. (A) Somatic metaphase chromosome spread and (B) Karyotype of female *Bufo regularis* specimen from Debre Zeit (DZ-C2).

Table 4. Karyological data of the studied amphibians from different localities in Ethiopia showing the different centromeric positions (M, m, sm, st, t, and T), the diploid (2n) and fundamental (FN) numbers.

Species	Specimen code	M	m	Sm	St	t	T	2n	FN
Rana angolensis	A-ST$_{(1)}$	-	6	16	2	2	-	26	50
	A-AF$_{(1)}$	-	6	16	2	2	-	26	50
Xenopus clivii	A-EK$_{(3)}$	-	14	6	16	-	-	36	72
Hyperolius spp.	A-EK$_{(10)}$	-	16	4	4	-	-	24	48
Ptychadena neumanni	Z-L$_{(2)}$	-	22	2	-	-	-	24	48
	D-T$_{(5)}$		22	2	-	-	-	24	48
Ptychadena cooperi	A-K$_{(2)}$	-	22	2	-	-	-	24	48
Bufo regularis	DZ-C$_{(2)}$	-	16	4	-	-	-	20	40
Bufo garmani	DZ-C$_{(8)}$		16	4	-	-	-	20	40
	DZ-C$_{(11)}$		16	4	-	-	-	20	40

NB: M and m = metacentric; sm = submetacentric; st = subtelocentric or acrocentric; t and T = telocentric

6. DISCUSSION

The chromosomes of eight anuran species, belonging to four families and five, from six different geographical areas in Ethiopia were studied and five different karyotypic forms were found. *Rana angolensis, Ptychadena neumanni, P. anchietae,* and *P. cooper* belong to family Ranidae; *Bufo garmani, and B. regularis* to Bufonidae; *Xenopus clivii* to Pipidae; and *Hyperolius* spp. to Hyperoliidae.

The chromosome complement of *Rana angolensis* is relatively asymmetrical and consists of two distinct classes of chromosome sizes: large, mainly sm, and small, which are sm, st, and t. It is known that telocentric can be derived by mis-division of meta- or submetacentric but there is no need to assume that it is necessarily derived. Increasing asymmetry may also result from pericentric inversions and unequal translocations (Stebbins, 1971).

The karyotype of *Rana angolensis* as observed in the present study is in agreement with the karyotype reported by Bogart and Tandy (1981). Both karyotypes share similar diploid number (2n = 26) and their relative size (five pairs of large and eight pairs of small) of chromosomes are similar. In spite of this similarity, however, differences were also detected, especially in the fundamental number. The disagreements were also found in the centromere position of some chromosomes, especially in pairs 4, 5, 6, 8, 9, 11, and 13, which are st, m, sm, m, st, and sm, respectively in Bogart and Tandy (1981); whereas these chromosomes are sm except pair 6 (st) and last pair (t) in this paper.

The presence of a small telocentric pair (pair 13) in the karyotype of *Rana angolensis* studied, unlike described in Bogart and Tandy (1981), is may be due to translocations. In this case, the chromosomal number remained unchanged (2n = 26), whereas the fundamental number is changed from 52 to 50. The differences in centromeric position (arm ratio) might also be explained in the following manner. By converting metacentric to telocentric chromosome, pericentric inversion can reduces the fundamental number of well-developed chromosome arms. According to Bogart (1974), there are similar reported cases of pericentric inversion in amphibian chromosomes. For instance, *Leptodactylidae andreae* has the same number of chromosomes as *L. hylaedactylus* but has only half as many pairs

of telocentric chromosomes. Such difference is evidently the result of pericentric inversions involving at least three pairs of chromosomes.

Morphological differences among individuals reflect differences in the product of genes modified by the environmental influences, while chromosomal difference reflects differences in the source of genetic variation. Variations observed in fundamental number and centromeric position reveal that these amphibians are diverse in their karyotypes. Chromosomal rearrangements may alter the number of chromosome, chromosome arms or both and centromeric position, so that such changes might have occurred in the evolution of chromosomes of the studied amphibians (Fredga, 1977)..

The diploid and fundamental numbers of all *Ptychadena* species: *P. neumanni, P. anchietae,* and *P. cooper*; examined in the present study are 24 and 48, respectively. The 2n and FN are in agreement with results of Bogart and Tandy (1981) on *P. anchietae*. Although chromosome sizes in these *Ptychadena species* vary from species to species with minor differences, they are karyotipically very similar in terms of relative size (two pairs of large, one pair of intermediate, and three pairs of small) and karyotypic formula (22 m + 2 sm). This is again not significantly different from the values obtained for *P. anchietae* by these authors, except one pair (m in this paper) but st in Bogart and Tandy (1981). This minor difference may be a result of different treatments with colchicine or hypotonic solution (Cuevas and Formas, 2003).

Being composed of all biarmed chromosomes, the karyotypes of all *Ptychadena* species examined are more or less symmetrical. According to White (1973), there are two possible explanations for this. The first possibility is that, this karyotype may not be derivative, so it might stem from some symmetrical karyotype of a remote ancestor. Another possibility is that metacentric chromosomes can arise by two by two fusions of telocentrics without any material alteration of either the chromosomal contents or the arrangement of genes.

Despite the fact that symmetrical karyotype is typical for the genus *Ptychadena*, there might have been minor structural differences among the karyotypes of different species. Such structural differences among morphologically uniform karyotypes might be revealed

through molecular or high-resolution cytogenetic techniques (Cuevas and Formas, 2003).

The diploid and fundamental numbers of the *Hyperolius* specimens from Addis Ababa (A-EK10) are 24 and 48, respectively. The chromosome set of the *Hyperolius* specimen possesses 8 pairs of metacentric, two pairs of submetacentric, and two pairs of subteocentric chromosomes. Being composed of bi-armed chromosomes, the karyotype is more of symmetrical in type.

It has not been possible to determine which species of the genus *Hyperolius* was used in the present study from external morphology, as the specimen was not fully developed to adult stage. Our chromosome data could help in this regard either. The 2n = 24 has been reported for five species (*Hyperolius concolir, H. mariae, H. nasutus, H. ocellatus, and H. steindachneri*) by Bogart and Tandy (1981), which is in agreement with our finding for the present specimen. Whether this specimen belongs to one of the above species or a different species need further detailed studies involving chromosomal and morphological characteristics.

The chromosome complement of *Xenopus clivii* is characterized by 2n = 36 and FN = 72, which is in agreement with that reported by Tymowska (1977). The karyotype is dominantly symmetrical, since composed of all bi-armed chromosomes, and in terms of chromosome size it is homogeneous.

The 2n = 20 and FN = 40 has been reported for both species of Bufo, *B. garmani* and *B. regularis*, by Bogart (1968) which is in agreement with our finding. Despite the fact that the present study shows karyotypic similarity and the application of karyotypic technique only did not permit differentiation of these *Bufo* species, there might exist minor structural differences that would be revealed through molecular or high-resolution cytogenetic techniques (Cuevas and Formas, 2003).

Although chromosome sizes of different species of a given genera vary from species to species with minor differences, they are karyotipically very similar in-terms of chromosome number and karyotypic formula so that differentiation of species in a given

genera based on their karyotype is very difficult. In addition, satellite and secondary constrictions were not detected. This is may be due to the nature of condensation of metaphase chromosomes and the small size of chromosomes of the studied amphibian. Satellites were better detected on late-prophase or pro-metaphase chromosome (Kifle Dagne and Heneen, 1992).

Size polymorphic pairs, the character of sex-chromosomes, are observed in male karyotype of *Rana angolensis* and in female karyotype of *Bufo regularis,* which is in disagrment with the reports of Bogart and Tandy (1981) and Bogart (1968), respectively. Whether this polymorphic pairs are really sex chromosomes or not, further detailed cytogenetic studies such as specific banding techniques are needed. There are similar reports of sex chromosome polymorphism in amphibians. For example, female heteromorphism and male heteromorphism had been reported in the karyotype of South African bull frog (*Pyxicephalus adspersus*) and *Rana esculenta*, respectively (Schimid, *et al*., 1990).

It is known that numerous chromosomal changes have occurred since the karyotype of the common ancestor of amphibians and resulted in a variety of karyotypes with diploid number ranging from 14 to 104 (Fredga, 1977). The chromosome complements described in this study can be grouped into two categories: symmetrical and asymmetrical karyotypes. There are two possible trends in karyotype evolution: symmetrical karyotype leads to asymmetrical karyotype or asymmetrical karyotype gives way to symmetrical karyotype (Zhu, *et al*., 2002). Even though there are two karyotypic forms, the direction of changes cannot be determined on the basis of these data.

According to Cuevas and Formas, (2003) centric fusion, fission, and translocation have been used to explain the evolution of frog's karyotype. Therefore, the symmetrical karyotypes observed in this study may be derived from asymmetrical karyotype through centric fusion of some telocentric chromosomes to give rise to meta- or submetacentric (bi-armed) chromosomes. However, there is a need to consider that these chromosome sets may not be derived and thus they might stem from some symmetrical karyotype of a remote ancestor. On the other hand, asymmetrical karyotypes may be evolved from symmetrical

ones since numerous telocentric chromosomes may be formed from bi-armed chromosomes by centric fission but there is no need to assume that all are necessarily derivative.

In the course of observation and specimen collection, it is found to be true that some of species of amphibians, which were previously, reported by Largen (2001) in streams in the capital Addis Ababa and other cities are not found. In addition, even those species collected are found up-streams near mountains from which the river starts but not down- streams crossing the city. These disappearances or a decrease in population of some previously reported species probably due to stream water contamination by stream flow of toilet and the widely social practicing of disposing all kinds of garbage in the rivers.

7. CONCLUSIONS AND RECOMMENDATIONS

7.1. CONCLUSIONS

In the present study, which attempted to determine the chromosome number, chromosome morphology and karyotype of some species of Ethiopian amphibians, revealed that the studied amphibian species have chromosome number ranging from 2n = 20 to 2n = 36. It also shows five karyotypic forms based on karyotypic formula and chromosome number. *Bufo garmani, and B. regularis,* 2n = 20 with 16 m + 4 sm; *Rana angolensis,* 2n = 26 with 6 m + 16 sm + 2 st + 2 t; *Ptychadena neumanni,* and *P. cooperi,* 2n = 24 with 22 m + 2 sm; *Xenopus clivii,* 2n = 36 with 14 m + 6 sm + 16 st; and *Hyperolius* spp., 2n = 24 with 16 m + 4 sm + 4 st karyotypic formula. Besides, size polymorphic pairs that are a characteristic of sex chromosome pair has been obtained in two species, *Bufo regularis* and *Rana angolensis.*

The karyotype of the studied amphibian species are dominantly symmetrical type**,** showing entirely bi-armed chromosomes of the set and larger number of metacentric chromosomes, except *Rana angolensis.* The karyotype of *Rana angolensis* is hetrogenous and largely asymmetrical with two distinct classes of chromosome size: five pairs of large and eight pairs of small chromosomes.

7.2. RECOMMENDATIONS

Based up on the present study on amphibian chromosome from six localities in Ethiopia, the following recommendation can be made:

- In view of the existence of a large number of endemic amphibians in this country more investigations are needed to be done on chromosomes of various species of amphibians from different localities of the country so as to document all the karyotypic forms.

- More advanced molecular cytogenetic techniques such as FISH and GISH would give valuable information about the number and location of nucleolar organizer regions and satellites as well as distribution of various classes of repetitive DNA sequence, which are important for differentiation of morphologically similar karyotypes.

- To confirm whether the size polymorphic pairs in karyotypes of *Rana angolensis* and *Bufo regularis* is really sex chromosomes or not, more advanced cytogenetic techniques are need to be done.

- To infer phylogenetic and evolutionary status of the species, an integrated approach of all available data from karyological, molecular and evolutionary studies are required.

Amphibians are probably among the most seriously threatened group of animals due to widespread environmental degradation by an ever-increasing human population (Largen, 2001). Based on personal observations at Addis Ababa and other towns, it is highly recommended that the society in general, and the concerned governmental and non governmental institutions in particular, should be aware and act against the consequences of human-induced streams and water contamination in order to insure the safeguard of amphibians and other aquatic life from extinction.

REFERENCES

Amphibian; From Wikipedia, the Free Encyclopedia, Retrieved from"http://en. wikipedia. org/wiki/Amphibian", last modified 20:04, 24 June 2007.

Beck, C. W. and Slack, J. M. W. (2001). An amphibian with ambition: a new role for *Xenopus* in the 21st century. *Genome Biology* **2**(10): 1029 – 1039.

Bogart, J. P. (1968). Chromosome number difference in the amphibian genus *Bufo*: The *Bufo regularis* species group. *Evolution* **22**: 42-45.

Bogart, J. P. (1974). A Karyosystematic study of frogs in the genus *Leptodactylus* (Anura: Leptodactylidae). *Copeia* **3:** 728-737.

Bogart, J. P. and Tandy, M. (1981). Chromosome lineages in African Ranoid frogs. *Ital. J. Zool*. **5:**55-91.

Bougourd, S. M. and Jones, R. N. (1997). B-chromosome: a physiological enigma. *New Phytol*. **137:** 43-54.

Camacho, J. P. M., Sharble, T. F. and Beukeboom, L. W. (2000). Chromosome evolution. *Phil. Trans. R. Soc*. **366**:163-178.

Clarke, B. T. (1997). The natural history of amphibian skin secretions, their normal functioning and potential medical applications. *Biol. Rev.* **72:** 365-379.

Cogger, H. G. (1986). *Reptiles and Amphibians of Australia*. 4th ed. Dai Nippon Printing Co. Ltd, Hong Kong.

Cuevas, C. C. and Formas, J. R. (2003). Cytogenetic analysis of four species of the genus *Alsodes* (Anura: Leatodactylidae) with comments about the karyological evolution of the genus. *Hereditas* **138**(2): 138-147.

Dietrich, A. J. (1986). The influence of fixation on the morphology of mitotic chromosomes. C*an. J. Genet. Cytol*. **28**:536-539.

Duellman, W. E. (1967). Additional studies of chromosomes of Anuran amphibians. *Syst. Zool.* **16**(1): 38-43.

Etheridge, R. E. (1996). Methods for preserving amphibians and reptiles. For scientific study. w.w.w. mfobrien@umich.edu.

Fredga, K. (1977). Chromosomal change in vertebrate evolution. **In**: *Turning Points in Zoological Science*, pp.377-397. The Royal Society, London.

Frog, From *Wikipedia, the Free Encyclopedia*, Retrieved from "http://en. wikipedia.org/ wiki/Frog", last modified 20:09, 2 June 2007.

Frost, D. R. (2007). *Amphibian Species of the World: An Online Reference*. Version 5.0 (1, February, 2007). Electronic Database accessible at, http://research. amnh.org/herpetology/amphibia/index.php.American Museum of Natural History, New York, USA.

Gibson, L. J. (1984). Chromosomal change in mammalian speciation: A literature review. *Origins* **11**: 67-89.

Griffiths, A. J. F., Miller, J. H., Suzuki, D. T., Lewontin, R. C., and Gelbart, W. M. (1999). *An Introduction to Genetic Analysis*. 7th ed. W. H. Freeman Pub. USA.

Gropp, A. and Winking, H. (1972). Robertsonian chromosomal variation and identification of metacentric chromosomes in feralmice. *Chromosoma* **39**: 265-288.

Gupta, P. H. (2004). *Cell and Molecular Biology*. 3rd ed. Rasdtogi, Pub., India.

Hasumi, M. (2003). About Hynobiidae. *Biglobe.* Electronic Database accessible at http://www5d.biglobe.ne.jp/~hasumi/doc1/hyno_e.html.(Accessed: 2003).

Hayes, T. B. (1998). Sex Determination and primary sex differntiation in amphibians: Genetic and Developmental Mechanisms. *Exper. Zoo.* **281**: 373-399.

Hsu, T. H. (1975). A possible function of constitutive hetrocromatin, the background hypotesis. **In**: Proceedings of the 13th International 'Congress of Genetics, Part II Symposia. *Genetics* **79**: 137-150.

Joeng, K. S., Song, E. J., Lee, K. J and Lee, J. (2004). Long lifespan in worms with long telomeric DNA. *Nature Genetics* **36**: 367-385.

Jones, R. N. (1995). B chromosomes in plants. *New Phytol*. **131**(85): 411-434.

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

Largen, M. J. (2001). Catalogue of the amphibians of Ethiopia, including a key for their identification. *Tropical Zoology* **14**: 307-402.

Larson, A., Wake, D., and Devitt, T. (2006). Caudata. Salamanders. Version 05 September 2006. http://tolweb.org/Caudata/14939/2006.09.05 in The Tree of Life Web Project, http://tolweb.org/

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

Marks, J. (1983). Rate of karyotype evolution. *Syst. Zool.* **32**(2): 207-209.

McClean, P. (1997). Eukaryotic chromosome structure. http://www.ndsu.edu/ instruct/mcclean/plsc431/eukarychrom/eukaryo3.htm.

Mertens, R. (1960). *The World of Amphibians and Reptiles*. Georg, G. Harrap and Co. Ltd.London.

Miller, J. J. (2003). "Amphibian Declines."http://www. livingunderworld. org/biology/caudata/

Myers, P., Espinosa, R., Parr, C. S., Jones, T., Hammond, G. S. and Dewey T. A. (2006). Order Anura (frogs and toads). *The Animal Diversity Web*. University of Michigan Museum of Zoology. Retrieved on 2006- 05-13.

Noble, G. K. (1954). *The Biology of Amphibian*. Devor Pub., Inc. New- York.

Orr, R. T. (1976). *Vertebrate Biology*, 4thed. W. B. Sanders Company, Philadelphia.

Price, S. (2003). "Amphibians and Reptiles: Herps." April 3, 2003 @2001 Cofrin Center for Biodiversity, University of Wisconsin 12-7-03 <www.uwgb.edu/biodiversity/ biota/herps/hibians.htm>

Robbins, L. W. and Baker, R. J. (1978). Karyotypic data for African mammals, with a description of an *in vivo* bone marrow technique. *Hereditas* **42**: 79 - 120.

Rocco, L. (2001). $(TTAGGG)_n$ telomeric sequence in selachian chromosomes. *Heredity* **87**(5): 583-588.

Ronne, M. (1989). Chromosome preparation and high-resolution R- and G-banding techniques. **In**: *Cytogenetics of Animals*, pp. 11-39, (Halnan, C. R. E., ed). C. A. B International, UK.

Saitoh, Y. and Laemmli, U. K. (1994). Metaphase chromosome structure: bands arise from a differential folding path of the highly AT-rich scaffold. *Cell* **76**: 609-622.

Schimid, M., Nando, I., Steinein, C., and Epplen, J. T. (1990). Evolution of sex chromosome in amphibian. **In**: *Chromosome Today* Vol. 10. London, UNWIN HYMAN.

Session, S. K. Mortiz, C. and Marble, B. K. eds (1996). *Molecular Systematics,* 2nded. Sinauer Associatiate Inc., Massachusetts, U.S.A.

Sharma, A. (1991). *Chromosomes*, 3rded. Oxford and IBH Publishing Co. Pvt. Ltd; New Delhi.

Stace, C. A. (2000). Cytology and cytogenetics as a fundamental taxonomic resource for the 20th and 21st centuries. *Taxon* **49:** 451 – 477.

Stebbins, G. L. (1971). *Chromosomal Evolution in Higher Plants*. Edward Arnold (Publishers) Ltd., London, pp. 1-20.

Steward, J. W. (1969). *The Tailed Amphibians of Europe*. Taplinger Pub. Co., Inc. New York.

Stewart, M. M. (1967). *Amphibians of Malawi*. State University of New-York Press. New York.

Touart, L. (2004). Amphibian Growth and Reproduction Assay (Tier 2). Battelle 505 King Avenue Columbus, Ohio 43201

Twyman, R. M. (2003). *Advanced Molecular Biology*, 3rd ed. Viva Books Pvt. Ltd; New Delhi.

Tymowska, J. (1977). A comparative study of the karyotypes of eight *Xenopus* species and subspecies possssing a 36 chromosome complement. *Cytogenetic Genome Res.* **18:** 165 - 181.

Vences, M. Köhler, J. Ziegler, T., and Böhme, W. (eds) (2006). The current status of genetic exploration in amphibians: taxonomic and geographical disparities. Herpetologia Bonnensis II. Proceedings of the 13th Congress of the Societas Europaea Herpetologica. pp.193-195

Weaver, R. F. and Hedrick, P. W. (1997). *Genetics*, 3rded. Wmc. Browen Pub. Dubuque.

White, M. J. D. (1978). *Modes of Speciation*. W. H. Freeman, San Francisco- California.

Zhu, B., Feng, Z., Qu, A., Gao, H., Zhang, Y., Sun, D., Song, W., and Saura, A. (2002). The Karyotype of the caudate amphibian *Andrias avidianus*. *Hereditas* **136**: 85-88.

APPENDICES

Appendix 1. Zoogeographic affinities of the Ethiopian amphibians and their altitudinal ranges within which they have been recorded in Ethiopia according to Largen, (2001).

NB: CAF = Central African forest, E = Endemic, EAM = East African mountain, EAS = East African savanna, EP = Ethiopian plateau, PAS = Pan-African Savanna, SA = Somali-arid, WAS = West African savanna, U = Unclassified.

FAMILY	GENUS & SPECIES	ALTITUDE RANGE	ZOOGEOGRAPHIC CATEGORY
Caeciliaidae	⇨***Sylvacaecilia***		
	S. grandisonae (Taylor 1970)	500-2150	E
Pipidae	⇨***Xenopus***		
	X. clivii (Peracca 1898)	820-2745	EP
	X. Largeni (Tinsley 1995)	2500-2650	E
Bufonidae	⇨***Bufo***		
	B. asmarae (Tandy *et al*.,1982)	(600?)2000-3000	EP
	B. blanfordii (Boulenger 1882)	380	SA
	B. dodsoni (Boulenger 1895)	380-1800	SA
	B. garmani (Meek 1897)	400-2000	U(SA?)
	B. kerinyagae (Keith 1968)	1500-3300	EAM
	B. langanoensis (Largen *et al*.1978)	700-1585	E?
	*B. lughensis (*Loveridge 1932)	400-1420	SA
	*B. maculatus (*Hallowell 1855)	500-1300	PAS
	*B. regularis (*Reuss 1834)	200-1500	U (PAS?)
	B. steindachneri (Pfeffer 1893)	3200-4000	WAS
	*B. xeros (*Tandy *et al*. 1976)	1950-3520	E
	⇨***Altiphrynoides***		
	A. malcolmi (Grandison 1978)	3200-4000	E
	⇨ ***Spinophrynoides***		
	S. osgoodi (Loveridge 1932)	1950-3520	E
Microhylidae	⇨***Balebreviceps***		
	B. hillmani Largen & Drewes 1989	3200	E
	⇨***Phrynomantis***		
	P. somalicus (Scortecci 1941)	1200	SA

Continued

FAMILY	GENUS & SPECIES	ALTITUDE RANGE	ZOOGEOGRAPHIC CATEGORY
Ranidae	⇨ ***Euphlyctis***		
	E. occipitalis (Günther 1859)	515-1400	PAS
	⇨ ***Conraua***		
	C. Beccarii (Boulenger 1911)	1100-2500	EP
	⇨***Tomopterna***		
	T. cryptotis (Boulenger 1907)	400-2500	PAS
	⇨ ***Rana***		
	R. angolensis(Bocage 1866)	1000-3100	EAM
	⇨ ***Hylarana***		
	H. Galamensis (Duméril &Bibron 1841)	500 ?	PAS SA
	⇨ ***Hildebrandtia***		
	H.macrotympanum(Boulenger 1912)	400-1800 2500-3100	EAS E
	⇨ ***Ptychadena***		
	P. anchietae (Bocage 1867)	1300-2500	E
	P. cooperi (Parker 1930)	800-1000	E?
	P. erlangeri (Ahl 1924)	1550	E
	P. filwoha (Largen 1997)	400-2000	PAS
	P. harenna (Largen 1997)	2000-3000	E
	P.mascareniensis (Duméril & Bibron 1841)	820-3800	E
	P. nana (Perret 1980)	1850	EAS
	P. neumanni (Ahl 1924)	1000-1850	U(PAS?)
	P. porosísima (Steindachner 1867)	450-500	PAS
	P. pumilio (Boulenger 1920)	900-1000	WAS
	P. schillukorum (Werner 1907)	1800-1850	E
	P. tellinii (Peracca 1904)		
	P. wadei (Largen 2000)	800-2500	EAS
	⇨ ***Cacosternum***		
	C. boettgeri (Boulenger 1882)	2400-3200	E
	⇨ ***Ericabatrachus***		
	E. baleensis (Largen 1991)	2600-2650	E
	⇨***Phrynobatrachus***	800-2800	EAM
	P. inexpectatus (Largen 2001)	400-2200	PAS
	P. minutus (Boulenger 1895)		
	P. natalensis (Smith 1849)		

Continued

FAMILY	GENUS & SPECIES	ALTITUDE RANGE	ZOOGEOGRAPHIC CATEGORY
Rhacophoridae	⇨ ***Chiromantis***		
	C. kelleri (Boettger 1893)	500-1400	SA
	⇨ ***Leptopelis***		
	L. bocagii (Günther 1864)	515-1900	EAS
	L. gramineus (Boulenger1898)	1900-3900	E
	L. Ragazzii (Boulenger 1896)	1930-3100	E
	L. susanae (Largen 1977)	2600-3000	E
	L. Vannutellii (Boulenger 1898)	1500-2200	E
	L. yaldeni (Largen 1977)	2000-2700	E
	⇨ ***Kassina***		
	K. Maculifer (Ahl 1924)	1400	SA
	K. senegalensis (Duméril & Bibron 1841)	515-2000	PAS
	K. somalica (Scortecci 1932)	960-1500	SA
	⇨ ***Paracassina***		
	P. Kounhiensis (Mocquard 1905)	(1300?)1980-3200	E
	P. obscura (Boulenger 1894)	820-3000	E
	⇨ ***Afrixalus***		
	A. Clarkeorum (Largen 1974)	820-1800	
	A. enseticola (Largen 1974)	1700-2750	E
	A. Quadrivittatus (Werner 1907)	515	E (CAF?)
Hyperoliidae	⇨ ***Hyperolius***		
	H. balfouri (Werner 1907)	450-820	WAS
	H. kivuensis Ahl 1931	500-820	EAS
	H. nasutus Günther 1864	515-2000	PAS
	H. viridiflavus (Duméril & Bibron 1841)	400-2400	U (EAS?)
Hemisidae	⇨ ***Hemisus***		
	H. marmoratus (Peters 1854)	500-1850	PAS
	H. microscaphus (Laurent 1972)	1500-2700	E

Appendix 2. Summary of sites of capture, number of male and females, number of metaphase plates studied.

Species	Sites of specimens collection	No. of amph.			No.of cells analyzed.	2n chr. no
		M	F	T		
Rana angolensis	• Addis Ababa, Selame Technical and Vocational Training Center. • Addis Ababa, Abyssinia Tea and Coffea Factory. • Addis Ababa, Entoto Kidane Mhiret Church.	1 1 - 	1 1 1	2 2 1	6 3 4	26 26 26
Xenopus clivii	• Addis Ababa, Entoto Kidane Mhiret Church	1	-	1	5	36
Ptychadena anchietae	• Goro, Goro River	-	1	1	5	24
Ptychadenae coorperi	• Addis Ababa, Kotebae	-	2	2	4	24
Ptychadenae neumanni	• Dessie, Tenta • Ziway, Ziway lake	2 2	- 2	2 4	8 7	24 24
Bufo regularis	• Debre Zeit, Cheleklaka lake	-	1	1	4	20
Bufo garamanni	• Debre Zeit, Cheleklaka lake	1	1	2	9	20
Hyperoliidae *	• Addis Ababa, Entoto Kidane Mhiret Church	-	-	1	3	24

*Sex unidentified because the specimen has not fully completed the tadpole stage.

Appendix 3. Analysis of haploid chromosome complement of karyotyped species.

NB: Length refers to the normalized length of the chromosome relative to the total haploid complement length.

Appendix 4. Chromosome measurement: Total length of each chromosome, arm lengths, arm ratio, centromeric position and normalized length of *Rana angolensis* (Male specimen).
Mag: 2200

Image resolution: 37.8 pixels per cm

Marking order	Rank	Length each	Normalized length	Long arm	Short arm	Arm Ratio (L/S)	Centromere position
1	1	5.90	150	3.30	2.60	1.27	m
2	2	5.69	145	3.09	2.59	1.19	m
3	3	4.98	127	3.17	1.81	1.75	sm
5	4	4.83	123	3.15	1.68	1.87	sm
4	5	4.27	109	2.69	1.57	1.70	sm
6	6	4.20	107	2.96	1.24	2.39	sm
8	7	4.09	104	2.82	1.27	2.23	sm
7	8	4.08	104	2.67	1.41	1.89	sm
11	9	4.00	102	2.58	1.42	1.82	sm
10	10	3.96	101	2.55	1.41	1.81	sm
9	11	3.10	79	2.40	0.70	3.43	st
12	12	2.67	68	2.01	0.66	3.05	st
16	13	2.27	58	1.53	0.74	2.07	sm
18	14	2.27	58	1.53	0.74	2.07	sm
22	15	2.20	56	1.32	0.72	2.06	sm
25	16	2.16	55	1.49	0.67	2.22	sm
19	17	2.04	52	1.39	0.65	2.14	sm
21	18	2.04	52	1.41	0.63	2.24	sm
15	19	1.80	46	1.02	0.78	1.31	m
13	20	1.79	46	1.05	0.74	1.42	m
20	21	1.75	45	1.24	0.51	2.43	sm
23	22	1.73	44	1.27	0.47	2.72	sm
24	23	1.67	43	0.86	0.81	1.06	m
17	24	1.69	43	1.03	0.62	1.66	m
26	25	1.65	42	N/A	N/A	N/A	t
14	26	1.53	40	N/A	N/A	N/A	t

Total for set: 78.44

Total for haploid set: 39.22

Appendix 5. Chromosome measurement: Total length of each chromosome, arm lengths, arm ratio, centromeric position and normalized length of *Rana angolensis* (Female specimen).

Mag: 2200

Image resolution: 37.8 pixels per cm

Marking order	Rank	Length each	Normalized length	Long arm	Short arm	Arm Ratio (L/S)	Centromere position
3	1	8.71	155	4.91	3.81	1.29	m
2	2	8.15	145	4.47	3.67	1.22	m
1	3	7.06	126	4.74	2.42	1.95	sm
5	4	6.95	124	4.55	2.40	1.90	sm
4	5	6.22	111	4.40	1.82	2.42	sm
6	6	6.22	111	4.38	1.84	2.33	sm
8	7	5.84	104	4.33	1.51	2.86	sm
9	8	5.70	102	4.20	1.50	2.80	sm
7	9	5.69	102	3.85	1.85	2.08	sm
10	10	5.65	101	3.58	2.07	1.73	sm
11	11	3.58	64	2.72	0.86	3.16	st
14	12	3.40	61	2.56	0.84	3.05	st
12	13	3.25	58	2.26	0.99	2.20	sm
18	14	3.19	57	2.22	0.97	2.29	sm
22	15	3.09	55	2.30	0.79	2.90	sm
24	16	3.02	54	2.25	0.77	2.92	sm
25	17	2.99	53	1.89	1.10	1.72	sm
17	18	2.96	53	1.97	0.99	1.99	sm
23	19	2.87	51	1.62	1.25	1.30	m
13	20	2.81	50	1.62	1.19	1.36	m
21	21	2.72	49	1.73	0.99	1.75	sm
16	22	2.63	47	1.67	0.96	1.74	sm
15	23	2.61	47	1.44	1.17	1.23	m
19	24	2.57	46	1.35	1.22	1.11	m
26	25	2.14	38	N/A	N/A	N/A	t
20	26	2.09	37	N/A	N/A	N/A	t

Total for set: 112.11

Total for haploid set: 56.05

Appendix 6. Chromosome measurement: Total length of each chromosome, arm lengths, arm ratio, centromeric position and normalized length of *Ptychadena neumanni* (Male specimen).

Mag: 2200

Image resolution: 37.8 pixels per cm

Marking order	Rank	Length each	Normalized length	Long arm	Short arm	Arm Ratio (L/S)	Centromere position
1	1	9.16	138	4.71	4.45	1.06	m
2	2	9.16	138	4.60	4.56	1.01	m
3	3	7.77	117	4.21	3.56	1.18	m
6	4	7.61	115	4.27	3.34	1.28	m
8	5	7.59	114	4.95	2.64	1.88	sm
4	6	7.43	112	4.81	2.62	1.83	sm
7	7	7.29	110	3.93	3.37	1.17	m
9	8	7.23	109	3.96	3.33	1.17	m
10	9	6.57	99	3.53	3.04	1.16	m
5	10	6.57	99	3.68	2.89	1.27	m
16	11	5.38	81	3.30	2.08	1.59	m
13	12	5.28	80	3.10	2.18	1.42	m
14	13	5.12	77	2.81	2.31	1.22	m
15	14	5.04	76	2.61	2.43	1.07	m
18	15	4.38	66	2.30	2.08	1.11	m
11	16	4.38	66	2.33	2.05	1.14	m
23	17	3.98	60	2.19	1.79	1.22	m
17	18	3.85	58	2.14	1.71	1.25	m
20	19	3.44	52	1.89	1.55	1.22	m
21	20	3.37	51	1.83	1.53	1.20	m
24	21	3.25	49	1.73	1.52	1.14	m
19	22	3.10	47	1.60	1.50	1.07	m
22	23	2.96	45	1.56	1.40	1.11	m
12	24	2.94	44	1.50	1.44	1.04	m

Total for set: 132.73

Total for haploid set: 66.36

Appendix 7. Chromosome measurement: Total length of each chromosome, arm lengths, arm ratio, centromeric position and normalized length of *Ptychadena neumanni* (Female specimen).

Mag: 2200
Image resolution: 37.8 pixels per cm

Marking order	Rank	Length each	Normalized length	Long arm	Short arm	Arm Ratio (L/S)	Centromere position
1	1	7.67	142	4.24	3.43	1.24	m
2	2	7.30	135	3.84	3.46	1.11	m
4	3	6.48	120	3.38	3.10	1.09	m
3	4	6.33	117	3.35	2.98	1.12	m
10	5	6.15	114	3.94	2.21	1.78	sm
6	6	6.11	113	3.93	2.18	1.81	sm
7	7	5.69	105	3.31	2.38	1.39	m
5	8	5.48	101	3.32	2.16	1.54	m
11	9	5.02	93	2.59	2.43	1.07	m
12	10	4.86	90	2.58	2.28	1.13	m
8	11	4.54	84	2.78	1.76	1.58	m
14	12	4.47	83	2.79	1.68	1.66	m
9	13	3.83	71	2.16	1.67	1.30	m
20	14	3.72	69	1.92	1.80	1.06	m
16	15	3.67	68	1.96	1.71	1.15	m
18	16	3.57	66	1.84	1.74	1.06	m
17	17	3.55	66	1.79	1.76	1.02	m
13	18	3.40	63	1.73	1.67	1.04	m
23	19	2.90	54	1.51	1.39	1.09	m
15	20	2.87	53	1.53	1.33	1.15	m
24	21	2.84	53	1.72	1.12	1.53	m
19	22	2.73	51	1.55	1.18	1.31	m
21	23	2.48	46	1.32	1.16	1.14	m
22	24	2.43	45	1.24	1.19	1.04	m

Total for set: 108.05

Total for haploid set: 54.02

Appendix 8. Chromosome measurement: Total length of each chromosome, arm lengths, arm ratio, centromeric position and normalized length of *Ptychadena cooperi* (Female specimen).

Mag: 2200

Image resolution: 37.8 pixels per cm

Marking order	Rank	Length each	Normalized length	Long arm	Short arm	Arm Ratio (L/S)	Centromere position
1	1	10.04	130	5.46	4.58	1.19	m
2	2	10.04	130	5.45	4.59	1.19	m
3	3	8.96	115	4.54	4.42	1.03	m
10	4	8.88	110	4.48	4.40	1.02	m
20	5	8.37	108	5.63	2.75	2.05	sm
7	6	8.30	107	5.50	2.80	1.96	sm
6	7	8.03	104	4.76	3.27	1.46	m
4	8	7.95	103	4.60	3.35	1.37	m
11	9	7.10	92	3.97	3.13	1.27	m
5	10	7.01	91	4.15	2.87	1.45	m
8	11	6.93	90	3.79	3.13	1.21	m
9	12	6.80	88	3.54	3.26	1.09	m
15	13	5.47	71	3.06	2.41	1.27	m
12	14	5.40	70	2.74	2.66	1.03	m
23	15	5.25	68	3.02	2.23	1.36	m
22	16	5.25	68	3.04	2.21	1.38	m
14	17	5.02	65	2.94	2.08	1.41	m
21	18	4.86	63	2.66	2.20	1.21	m
13	19	4.71	61	2.81	1.90	1.48	m
16	20	4.64	60	2.67	1.96	1.36	m
19	21	4.13	53	2.09	2.04	1.02	m
17	22	3.94	51	2.06	1.88	1.10	m
18	23	3.74	48	1.88	1.86	1.01	m
24	24	3.64	47	2.02	1.62	1.25	m

Total for set: 154.44
Total for haploid set: 77.22

Appendix 9. Chromosome measurement: Total length of each chromosome, arm lengths, arm ratio, centromeric position and normalized length of *Bufo garmani* (Male specimen).

Mag: 2200

Image resolution: 37.8 pixels per cm

Marking order	Rank	Length each	Normalized length	Long arm	Short arm	Arm Ratio (L/S)	Centromere position
3	1	10.76	175	5.97	4.76	1.25	m
1	2	10.76	175	5.95	4.81	1.24	m
2	3	10.70	174	5.46	5.25	1.04	m
4	4	10.62	173	5.55	5.07	1.09	m
5	5	8.75	141	4.93	3.82	1.29	m
12	6	8.61	140	4.81	3.80	1.27	m
7	7	7.77	126	4.92	2.85	1.73	sm
13	8	7.77	126	5.02	2.75	1.83	sm
6	9	6.09	99	3.06	3.03	1.01	m
10	10	6.03	98	3.22	2.81	1.15	m
16	11	5.47	89	3.28	2.19	1.50	m
18	12	5.35	87	3.26	2.09	1.56	m
8	13	3.20	52	1.75	1.45	1.21	m
9	14	3.20	52	1.76	1.44	1.22	m
15	15	3.12	51	1.89	1.23	1.54	m
17	16	3.10	50	1.88	1.22	1.54	m
11	17	3.08	50	1.57	1.51	1.04	m
14	18	2.95	48	1.57	1.38	1.14	m
20	19	2.91	47	1.92	0.99	1.94	sm
19	20	2.71	44	1.79	0.92	1.95	sm

Total for set: 123.00
Total haploid for set: 61.50

Appendix 10. Chromosome measurement: Total length of each chromosome, arm lengths, arm ratio, centromeric position and normalized length of *Bufo garmani* (Female specimen).

Mag: 2200

Image resolution: 37.8 pixels per cm

Marking order	Rank	Length each	Normalized length	Long arm	Short arm	Arm Ratio (L/S)	Centromere position
1	1	9.13	179	4.86	4.27	1.14	m
3	2	8.40	164	4.37	4.03	1.08	m
4	3	7.90	155	4.10	3.81	1.08	m
2	4	7.87	154	4.40	3.47	1.27	m
7	5	6.97	136	3.93	3.04	1.29	m
5	6	6.25	122	3.66	2.59	1.41	m
6	7	6.13	120	3.91	2.22	1.76	sm
8	8	5.97	117	3.78	2.19	1.73	sm
10	9	4.92	96	2.60	2.32	1.12	m
9	10	4.92	96	2.60	2.32	1.12	m
11	11	4.87	95	2.67	2.20	1.21	m
12	12	4.77	93	2.48	2.29	1.08	m
14	13	3.93	77	2.24	1.69	1.33	m
17	14	3.38	66	2.00	1.38	1.45	m
19	15	3.07	60	1.54	1.33	1.01	m
18	16	3.07	60	1.57	1.50	1.05	m
15	17	2.78	54	1.60	1.18	1.36	m
20	18	2.78	54	1.60	1.18	1.36	m
16	19	2.59	51	1.67	0.92	1.82	sm
13	20	2.54	50	1.61	0.93	1.73	sm

Total for set: 102.25

Total haploid for set: 51.13

Appendix 11. Chromosome measurement: Total length of each chromosome, arm lengths, arm ratio, centromeric position and normalized length of *Bufo regularis* (Female specimen).

Mag: 2200

Image resolution: 37.8 pixels per cm

Marking order	Rank	Length each	Normalized length	Long arm	Short arm	Arm Ratio (L/S)	Centromere position
5	1	8.77	170	4.68	4.09	1.14	m
1	2	8.76	169	4.75	3.97	1.20	m
3	3	8.41	163	4.25	4.16	1.02	m
4	4	8.36	162	4.65	3.71	1.25	m
6	5	7.89	153	4.65	3.24	1.44	m
2	6	7.84	152	4.75	3.09	1.54	m
9	7	5.11	99	3.28	1.83	1.79	sm
8	8	5.11	99	3.23	1.88	1.76	sm
7	9	5.06	98	2.77	2.29	1.21	m
10	10	5.06	98	2.56	2.50	1.02	m
11	11	4.94	96	2.87	2.07	1.39	m
14	12	4.85	94	2.98	1.87	1.59	m
16	13	4.02	78	2.01	2.01	1.00	m
17	14	2.99	59	1.52	1.48	1.03	m
12	15	2.94	57	1.58	1.36	1.16	m
19	16	2.92	57	1.60	1.32	1.21	m
13	17	2.55	49	1.38	1.17	1.18	m
15	18	2.53	49	1.41	1.12	1.26	m
18	19	2.38	46	1.50	0.88	1.70	sm
20	20	2.10	41	1.33	0.77	1.73	sm

Total for set: 103.20
Total for haploid set: 51.60

Appendix 12. Chromosome measurement: Total length of each chromosome, arm lengths, arm ratio, centromeric position and normalized length *Hyperolius* spp.

Mag: 2200

Image resolution: 37.8 pixels per cm

Marking order	Rank	Length each	Normalized length	Long arm	Short arm	Arm Ratio (L/S)	Centromere position
2	1	9.89	156	5.30	4.58	1.16	m
1	2	9.42	148	5.27	4.14	1.27	m
3	3	8.08	127	5.34	2.74	1.95	sm
6	4	7.64	120	4.99	2.65	1.88	sm
5	5	7.40	117	5.90	1.50	3.93	st
7	6	7.18	113	5.71	1.47	3.89	st
8	7	7.16	113	5.47	1.69	3.24	st
4	8	7.13	112	5.76	1.37	4.20	st
10	9	5.99	94	3.86	2.13	1.82	sm
11	10	5.20	82	3.44	1.76	1.95	sm
16	11	4.67	74	2.39	2.29	1.04	m
9	12	4.59	72	2.33	2.26	1.03	m
15	13	4.49	71	2.51	1.98	1.27	m
20	14	4.42	70	2.58	1.84	1.40	m
13	15	4.25	67	2.29	1.96	1.17	m
14	16	4.07	64	2.19	1.90	1.15	m
18	17	3.60	57	1.88	1.72	1.09	m
17	18	3.57	56	1.90	1.67	1.14	m
12	19	3.57	56	2.05	1.52	1.35	m
19	20	3.42	54	1.72	1.70	1.01	m
24	21	3.07	48	1.56	1.51	1.04	m
22	22	2.93	46	1.57	1.36	1.15	m
23	23	2.70	43	1.57	1.13	1.38	m
21	24	2.53	40	1.45	1.08	1.34	m

Total for set: 127.00
Total haploid for set: 63.50

Appendix 13. Chromosome measurement: Total length of each chromosome, arm lengths, arm ratio, centromeric position and normalized length of *Xenopus clivii* (Male specimen).

Mag: 2200

Image resolution: 37.8 pixels per cm

Marking order	Rank	Length each	Normalized length	Long arm	Short arm	Arm Ratio(L/S)	Centromere position
8	1	6.93	74	4.07	2.85	1.43	m
1	2	6.89	73	4.21	2.67	1.58	m
18	3	6.76	72	3.94	2.82	1.40	m
2	4	6.75	72	3.73	3.02	1.23	m
12	5	6.50	69	3.82	2.67	1.43	m
7	6	6.25	67	3.75	2.50	1.50	m
3	7	6.24	67	4.10	2.14	1.92	sm
17	8	6.14	66	4.35	1.79	2.42	sm
26	9	5.90	63	3.80	2.10	1.81	sm
33	10	5.90	63	3.75	2.15	1.75	sm
9	11	5.68	61	2.98	2.70	1.11	m
14	12	5.64	60	3.37	2.27	1.48	m
27	13	5.59	60	4.21	1.38	3.05	st
15	14	5.48	59	4.40	1.08	4.07	st
19	15	5.25	56	3.95	1.30	3.04	st
32	16	5.21	56	3.91	1.30	3.01	st
16	17	5.17	55	4.38	0.79	5.54	st
28	18	5.13	55	4.39	0.74	5.93	st
13	19	5.12	55	4.06	1.06	3.83	st
5	20	5.10	54	4.19	0.91	4.60	st
36	21	5.09	54	3.68	1.41	2.61	sm
31	22	5.05	54	3.49	1.56	2.24	sm
11	23	4.81	51	3.69	1.12	3.28	st
25	24	4.72	50	3.70	1.02	3.63	st
4	25	4.69	50	3.71	0.98	3.79	st
6	26	4.66	50	3.70	0.96	3.85	st
30	27	4.59	49	3.59	1.00	3.59	st
35	28	4.44	47	3.53	0.95	3.88	st
10	29	4.35	46	2.73	1.62	1.69	m
20	30	4.27	46	2.58	1.69	1.53	m
34	31	4.23	45	2.62	1.61	1.63	m
29	32	4.12	44	2.57	1.55	1.66	m
22	33	4.09	44	2.42	1.67	1.45	m
24	34	3.66	39	2.00	1.66	1.21	m
23	35	3.56	38	2.76	0.80	3.45	st
21	36	3.42	37	2.58	0.84	3.08	st

Total for set: 187.416

Total haploid for set: 93.71

Appendix. 3 Analysis of haploid chromosome complement karyotyped species.

NB. Length refers to the normalized length of the chromosome relative to the total haploid complement length.

Species	Charac-ters	Chromosome pair																
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
.Bufo garamani -Male -Female	Length	175	173.5	146	126	98.5	87.5	52	50.5	49	45.5								
	Arm ratio	1.25	1.07	1.28	1.78	1.08	1.53	1.22	1.54	1.09	1.95								
	C. position	m	m	m	sm	m	m	m	m	m	sm								
	Length	172	154.5	129.5	118.5	96	94	71.5	60	54	50.5								
	Arm ratio	1.11	1.18	1.35	1.75	1.12	1.15	1.39	1.03	1.36	1.78								
	C. position	m	m	m	sm	m	m	m	m	m	sm								
.Bufo regularies	Length	169.5	162.5	152.5	99	98	95	68.5	57	49	43.5								
	Arm ratio	1.17	1.14	1.49	1.76	1.12	1.49	1.02	1.19	1.22	1.72								
	C. position	m	m	m	sm	m	m	m	m	m	sm								
.Ptychadenae neumani -Male -Female	Length	138	116	113	109.5	99	80.5	76.5	66	59	51.5	48	44.5						
	Arm ratio	1.04	1.23	1.86	1.17	1.22	1.51	1.15	1.13	1.24	1.21	1.11	1.08						
	C. position	m	m	sm	m	m	m	m	m	m	m	m	m						
	Length	138.5	118.5	109.5	107	92	83.5	70	67	64.5	53.5	52	45.5						
	Arm ratio	1.18	1.11	1.80	1.47	1.10	1.62	1.18	1.11	1.03	1.12	1.42	1.09						
	C. position	m	m	sm	m	m	m	m	m	m	m	m	m						
.Ptychadenae coorpori	Length	130	115.5	107	103.5	91.5	89	70.5	68	64	60.5	52	47.5						
	Arm ratio	1.19	1.03	2.01	1.42	1.36	1.15	1.15	1.37	1.31	1.42	1.06	1.13						
	C. position	m	m	sm	m	m	m	m	m	m	m	m	m						
Hyperolidus	Length	152	122	116.5	112.5	88	73	70.5	65.5	56.5	55	47	41.5						
	Arm ratio	1.22	1.92	3.91	3.72	1.89	1.04	1.34	1.16	1.12	1.18	1.10	1.36						
	C. position	m	sm	st	st	sm	m	m	m	m	m	m	m						
.Rana angolensis - Male - Female	Length	147.5	125	108	104	101.5	73.5	58	55.5	52	46	44.5	43	41					
	Arm ratio	1.25	1.81	2.05	2.56	1.82	3.24	2.07	2.14	2.19	1.37	2.58	1.36	N/A					
	C. position	m	sm	sm	sm	sm	st	sm	sm	sm	m	sm	m	t					
	Length	150	125	111	103	101.5	62.5	57.5	54.5	53	49.5	48.5	47	37.5					
	Arm ratio	1.26	1.93	2.40	2.83	1.91	3.11	2.29	2.91	1.86	1.33	1.75	1.17	N/A					
	C. position	m	sm	sm	sm	sm	st	sm	sm	sm	m	sm	m	t					
Xenopus clivii	Length	73.5	72	68	66.5	63	60.5	59.5	56	55	54.5	54	50.5	50	48	46	44.5	41.5	37.5
	Arm ratio	1.51	1.32	1.47	2.17	1.78	1.30	3.56	3.03	5.74	4.22	2.43	3.46	3.82	3.74	1.61	1.65	1.33	3.27
	C. position	m	m	m	sm	sm	m	st	st	st	st	sm	st	st	st	m	m	m	st