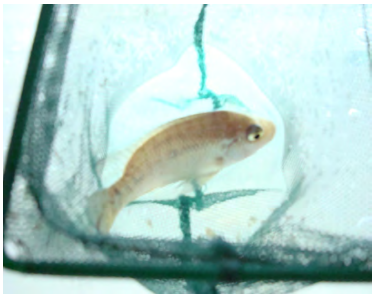
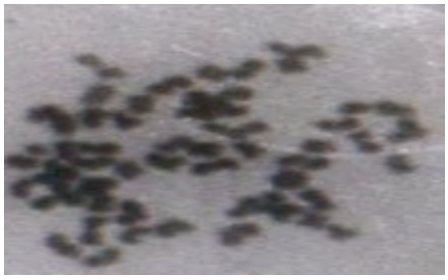
ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
DEPARTMENT OF BIOLOGY

Chromosome Study of Some Fish Species from rift valley Lakes and some other water bodies of Ethiopia





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By

FEKADU WORKU G/MARIAM

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ABBREVIATIONS

BG-oreo = *Oreochromis* specimens from LakeBabogaya

BG-zil = *Zilli* specimens from Lake Babogaya

Cha-syn = *Synodontis* specimens from LakeChamo

Cha-cla = *Clarias* specimens from LakeChamo

Cha-oreo = *oreochromis* specimens from Lake Chamo

Cha-hydro = *Hydrocynus* specimens from Lake Chamo

Cha-bar = *Barbus* specimens from Lake Chamo

Seb-oreo = *Tilapia* specimens from Sebeta artificial pond

Seb-goldfish = *Goldfish* specimens from Sebeta artificial pond

Zw-oreo = *oreochromis* specimens from Lake Ziway

ZW-zil = *Zilli* specimens from Lake Ziway

ABSTRACT

Fishes are the most diverse of the major vertebrate groups. However, cytogenetic studies in fish have not been well studied globally as well as here in Ethiopia. Five species belonging to four families were sampled randomly from rift valley lakes and some water bodies; and their mitotic chromosomes have been studied or determined using splashing method. For chromosome preparation, each live fish specimen was injected with colchicine (0.5% of 0.8/100 gm body weight) and kept alive for 6-8 hours. The gill filaments were removed and kept in hypotonic solution (0.75M KCL) for about 30 minutes and then were fixed in 3:1(methanol: glacial acetic acid) for 30 minutes. Gill filaments were grinded with 1-1.5 ml of fixative solution and filtered with gauze. Finally the cell solution centrifugation for 5 minutes and air-dry slide preparation was made and stained in Giemsa. Photographs were taken from the best metaphase plates of each specimen and chromosome numbers were determined. The result showed that, the chromosome numbers of (2n) obtained for *Tilapia zilli* (2n=44), *Oreochromis niloticus* (2n=44), *Clarias gariepinus* (2n=56), *Synodontis schall* (2n=54), and *Carassius auratus* (2n= 94) all except the last fish specimen are in agreement with the previous reports. Since karyotypic analysis could not be given to all species under the present study, it is better to carry out further cytological study in the future to get more reliable chromosome data for better understanding about their taxonomic and evolutionary relationships.

Key Words: - Fish, *Tilapia, Oreochromis, Clarias, Synodontis, Carassius*, diploid chromosome number, Rift Valley lakes, Ethiopia.

1. INTRODUCTION

Fishes are the most numerous and diverse of the major vertebrate groups. They dominate the waters of the world through a marvelous variety of morphological, physiological, and behavioral adaptations. Nelson (1994) has described over 24500 extant fish species and the number may eventually increase to about 28500. About 28,900 species of fish were listed in Fish Data Base in 2005, but some experts feel that the final total may be considerably higher. Freshwater fishes comprise until now, almost 13,000 species and 2,513 genera (including only freshwater and strictly peripheral species), or about 15,000 if all species occurring from fresh to brackish waters are included (Springer, 2007). Noteworthy is the fact that the estimated 13,000 strictly freshwater fish species live in lakes and rivers that cover only 1% of the earth's surface, while the remaining 16,000 species live in salt water covering a full 70% of the earth's surface. While freshwater species belong to some 170 families (or 207 if peripheral species are also considered), the bulk of species occur in a relatively few groups: the Characiformes, Cypriniformes, Siluriformes, and Gymnotiformes, the Perciformes (notably the family Cichlidae), and the Cyprinodontiformes. Biogeographically, the distribution of strictly freshwater species and genera are 705 genera with 4,035 species in the Neotropical region, 390 genera and 2,938 species in the Afrotropical, 440 genera and 2,345 species in the Oriental, 380 genera 1,844 species in the Palaearctic, 298 genera 1,411species in the Nearctic, and 94 genera 261 species in the Australian region (Springer, 2007).

Moyle and Cech (1988) reported that in Africa there are about 2000 freshwater species of fishes. The Great African lakes each contain more fish species than any other lake in the world (Freyer and Iles, 1972; Ribbink, 1991). According to Abebe Getahun (2005), about 153 valid species and sub-species of fishes, belonging to 12 orders and 24 families are found in Ethiopian freshwater. Golubstov and Mina (2003) reported 168 to 185 valid species of fish as occurring in Ethiopian waters. There are also about 10 exotic fish species introduced into Ethiopian freshwaters (Shibru Tedla and Fisseha H/Meskel, 1981). The number of endemic species of the country is estimated to range from 37 to 57 (Golubstov and Mina, 2003).

Fishes are found throughout the world, from altitudes more than 5 km above sea level as in Lake Titicaca located 3.8 km above sea level in the Andes to a depth of about 10 km in the Pacific Ocean (Bond, 1996). Some fishes like certain killfishes (Cyprinodon) inhabit hot springs, where the water temperature may reach 45^0C; others, like the icefishes, *Chaenocephalus,* are found in Antarctic seas, where water temperature may fall below 0^0 C (Larger, 1962). Some fishes can tolerate high salinity levels. For example, some species of gobies can tolerate salinity as high as 60 ppt (Nikolsky, 1978), noting that sea water has average salinity level of 35 ppt (part per thousands).

On the basis of feeding habits, fish can be detritivores, herbivores, carnivores and omnivores. Within these categories, fish can be characterized further as euryphagous, having a mixed diet; stenophagous, eating a limited assortment of food types; monophagous consuming only one sort of food. A majority of fish, however, are euryphagous carnivores. Often the feeding mode and food types are associated with the body form and digestive system (Helfman *et al*., 1997)

Fishes are well known for their high potential fecundity, and most species utilize the primitive condition of realizing thousands to millions of eggs annually (Hoar, 1969). Some fishes exhibit interesting reproductive behavior including their ability to care for their offsprings. Blumel (1979) confirmed parental care among 80 families of bony fishes that include guarding of eggs followed by nest building or cleaning of spawning substratum and fanning eggs, internal gestation, oral brooding, burying of eggs, and splashing water on egg deposited above the water line. Bond (1996) reported eggs are incubated intestinally by the cat fish, *Tachsuns* (*Ariid*).

Fishes are economically important for aquaculture, fish food, fishing, fish farming, recreation, angling, fish keeping and sport fishing. Worldwide, more than 100 million metric tons of bony fishes are harvested every year for human consumption. In some countries, bony fishes may provide up to 50% of all animal protein consumed (Bond, 1996). Bony fishes are also used to feed livestock, make fertilizer, and produce fish oil. Metallic paints, leather, glue, and medicines all are made from bony fishes or bony fish

byproducts (Bond, 1996). Some commercial fishing operations catch large numbers of non-targeted species, which are discarded.

As of 2006, the IUCN (International Union for Conservation of Nature) Red list describes 1173 species of fish as being threatened with extinction as a result of over fishing, habitat destruction (by pollution, dams, etc) and exotic species.

Cytogenetically, every organism is characterized by its own specific karyotype both in number and morphology. However, some reports indicate that a karyotypic variation may occur in different individuals of the same species (Nogusa, 1960). The possibility of using karyotype as racial markers has been suggested by Roberts (1967).

Cytogenetic studies in fish have not been comprehensive when compared to other vertebrate groups (Philips, 2001). Standard karyotypes have been reported in less than 10% of the more than 20000 species of fish. Karyptypes have been reviewed for about 1300 freshwater and saltwater fish species (Al-Sabti, 1987). A few studies have used fish standard karyotypes to examine taxonomic or systematic problems (Bolla, 1987). Fish chromosome data have great importance in studies concerning evolutionary systematics, aquaculture and mutagenesis (Al –Sabti, 1991).

Most of the fish species, especially within a genus have almost the same body structures. As a result, fish classification and phylogenetic studies have become complicated for many years. Currently, chromosomal taxonomy can be quite useful, both in determining the phylogenetic relationships of the taxa, as well as in the segregation of siblings or cryptic species (Kapoor, 2001). Except for the report by Golubstov (1996) and Berrebi (1998) no cytogenetic data on fishes of the Ethiopian freshwater have been available.

Therefore, the aim of this study is to provide chromosomal data on some Rift Valley lake fish species from Ethiopia, which contribute useful information about the diversity and status of fishes in the country.

2. General Descriptions of the Rift Valley Lakes

The Rift Valley lakes are a group of lakes formed by the East African Rift which runs through the whole eastern side of the African continent from the north to south. These lakes include some of the oldest, largest and deepest lakes in the world and many are freshwater eco-regions of great biodiversity, while others are alkaline or soda lakes supporting highly specialized organisms. (Http://en. Wijipedia.org/wiki/ Rift Valley Lakes, 2007).

The Rift Valley lakes are well known for the evolution of at least 800 cichlid species that live in their waters. More species will be discovered. The East African Rift came into being about 40 million years ago as the African tectonic plate began to split. Lakes such as Lake Malawi and Lake Tanganyika have formed in the various valleys of the rift zone including the huge Lake Victoria (Snoeks, 2000).

Lake Tanganyika is the oldest of the Rift Valley lakes which is about 8-10 million years old. Several lines of evidence suggest that Lake Tanganyika acted as evolutionary reservoir, seeding the surrounding lakes and river systems to initiate new species flocks within the last 1-2 million years (Salzburger *et al*., 2005).

The Ethiopian Rift Valley lakes are the northern most of the African Rift Valley lakes. In central Ethiopia, the Great Rift Valley splits the Ethiopian highlands into the northern and southern halves, and the Ethiopian Rift Valley lakes occupy the floor of the rift valley between the two highlands. Most of the Ethiopian Rift Valley lakes do not have an outlet, and most lakes are alkaline. (Http: //en. Wijipedia.org/wiki/ Rift Valley Lakes, 2007).

2.1. The Diversity of Fish Fauna in the Ethiopian Rift Valley Lakes

The Rift lakes from which greater parts of the country's fish harvest is coming (LFDP, 1996), are found within the Ethiopian Rift Valley. Based on the similarities of their fish fauna, these lakes can conveniently be categorized into the southern lakes (Lakes Chamo, Abaya, and Chew-Bahir), the northern lakes (Lakes Awassa, Shalla, Abijata, Langano and Ziway) (LFDP, 1996) and extreme northern saline lakes (Lakes Afambo, Gamari, Afdera, Asale and parts of Abbe).

The diversity and distribution of fish species recorded from the Ethiopian Rift Valley Lakes have been reported by several authors (Abebe Getahun, 2001; Golubstov *et al*., 2002; Golubstov and Mina 2003). From the Rift Valley lakes of Ethiopia, a total of 31 native and 4 introduced fishes have been described (Golubstov *et al*., 2002). The southern Rift Valley lakes, including Lakes Abaya, Chamo, and Chew-Bahir basin, contain about 23 native fish species in contrast with only 12 such species recorded from central and northern part of the Valley (Golubstov *et al*., 2003). This higher species diversity in southern basin has been attributed to the presence of taxa of Nilotic and East African origin (Golubstov *et al*., 2002).

The fish fauna of the Ethiopian Rift Valley are naturally impoverished but at the same time it includes relatively high number of the Ethiopia's endemic, *Barbus ethiopicus* and *Garra makiensis* from Lake Ziway and Awash basin, *Danakilia franchetti* and newely described *Lebias stiasssnyae* from Lake Afdera (Golubstov *et al*., 1996).

2.2. General Description of the Fish Species under Study.

2.2.1. Family Cichlidae.

The cichlid family is characterized by the presence of ctenoid or cycloid scales; and head not completely covered by scales. In this family the mouth is protractile; teeth variable in form and number. The family is distinguished by the presence of well developed

pharyngeal teeth, carried on a triangular bone. Members of this family have single nostril on each side; and lateral line interrupted, usually into two parts (Shibru Tedlla, 1973).

Cichlids are freshwater and brackish water fishes inhabiting Africa, South America, Central America and parts of Asia and North America. Their center of biodiversity is located in the East African Great Lakes (Victoria, Malawi, and Tanganyika), that harbor more than two thirds of the estimated species in the family (Snoeks, 2000). Several lines of evidence suggest that functional divergence in feeding morphology has contributed to the radiation and maintenance of cichlid species diversity. Adaptive variation in jaw shape is critical to the success of this group (Albertson and Kocher, 2006).

Cichlids have been as model organisms to study diversity of evolutionary trends such as parental care, mating systems, sexual selection, and functional morphology (Keenleyside, 1991). Cichlid fishes are important evolutionary model (Kocher, 2004). Their uncanny ability to exploit new environments by radiating into a wide array of shapes and forms has made them to study the factors that underlie morphological diversity.

Moreover, because of the recent origin of most cichlid radiations, taxa that differ dramatically in shape can be crossed to produce viable and fertile hybrids. This offers a unique opportunity to study the genetic basis of characters that afford species an adaptive advantage. There are also multitudes of genomic resources available to cichlid researchers, including genetic linkage maps, physical maps, BAC libraries, and cDNA resources including micro arrays (Katagiri *et al*., 2005). Importantly, the close evolutionary relationship among African cichlids ensures that genomic resources developed in one species may be used for studies in thousands of related taxa (Albertson and Kocher, 2006).

2.2.1.1. *Tilapia zilli*

This is an introduced fish species into Ethiopia. The species possesses eight to twelve gill rakers on the lower part of the first gill arch and deep body. The mean number of spines in the dorsal fin is 15. The species is distinguished by its six or seven dark vertical bars,

of variable intensity, may be visible on the body and caudal peduncle. Dorsal, caudal, and anal fins are olivaceous with yellow spots, the dorsal and anal fins often outlined by a narrow orange band. ‘‘The tilapia mark’’ is a large, black, nearly circular spot almost completely outlined in yellow. The coloration of breeding fishes is more intense than that of non –breeders. This fish species is distributed in Lake Chamo and Abaya (Shibru Tedlla, 1973).

The reported chromosome number count showed that *Tilapia zilli* has diploid with chromosome number 2n=44 Lévéque (1997).

2.2.1.2. *Oreochromis niloticus* Linneaus, (1757)

This fish species is characterized by caudal fin with numerous vertical and wavy bars. There are usually 22 gill rakers on the lower part of the gill arch. Lateral line series with 28-30 (usually 29) scales. Body color is with faint traces of six or seven dark vertical bars on the flanks and caudal peduncle. Dorsal and anal fins are grayish; somewhat irregularly spotted; caudal fin gray, with numerous dark-red, wavy vertical bars. In breeding males, the vertical surface of the body, the anal, dorsal, and pelvic fins are black; and the head and flanks are flushed with red (Shibru Tedlla, 1973).

According to Shibru Tedlla (1973) this species is distributed in Lake Tana, Baro River, Lakes Ziway, Abaya, Langano, Abijata, Chamo, and Awash River, Suksuk River, Mojo River (Wabi Shebeli system), Ergino River (Omo system).

The reported chromosome count in the species shows diploid number of chromosome 2n=44 Lévéque (1997).

2.2. Family Claridae

The fishes of this family are characterized by a large armored head, no spine in the dorsal fin which has a long base, the presence or absence of a long adipose fin, a long anal fin and a superbranchial organ for breathing. They have four pairs of unbranched circum-oral barbells. The clarids are widely distributed in Africa, Syria and South –East Asia. Two genera occur in Ethiopia with four species (Shibru Tedlla, 1973).

2.2.2.1. *Clarias gariepinus*

This fish species is distinguished by its large bony head with small eyes. Dorsal and anal fins are long. No adipose fin. Pectoral fin with stout, serrated spine, used for defense or ‘‘walking’’ overland. Mouth is terminal and large. Four pair of barbells is present. Color varies from sandy yellow through gray to olive with dark greenish brown markings. Well developed supra-branchial organ is present (Skelton, 1970)

Reported chromosome number of *Clarias gariepinus* shows a diploid chromosome of number 2n=56 Lévéque (1997).

2.2.3. Family Mochokidae

Amongst the 10 freshwater cat fish families occurring in Africa, Mochokidae is one of the five endemic families to this continent (Skelton, 2001). It is the largest African family with 10 genera and approximately 170 species. The fishes of the family Mochokidae are characterized by scaleless body, head heavily armored; dorsal fin short with well developed spine. Adipose and dorsal fins are long. Anal fin is short. Two genera have been recorded from Ethiopia (Shibru Tedlla, 1973).

2.2.3.1. *Synodontis schall* (Bolch-Schneider, 1801)

The species is recognized by the presence of outer mandibular barbells with slender branches, the inner pair with somewhat stouter branches. Dorsal fin is consisting of a spine and 6-7 branches rays. Pectoral fins granulate on its interior face, strongly serrated on the posterior face. The color of this species is olivaceous above, lighter; irregular spots occur on the body (Shibru Tedlla, 1973).

The reported chromosome number of this species shows a diploid chromosome number of 2n=54 Lévéque (1997).

2.2.4. Family Cyprinidae

The cyprinidae is one of the most wide spread species in freshwater and possibly the largest family of freshwater vertebrates (Coad, 2005). This family is distributed in North America, Africa, Europe, and Asia. Its highest diversity is found in Asia, where over 2100 species or almost 10% of the world fish is found (Coad, 2005). The family is characterized by cycloid scales, jaws completely devoid of teeth, no adipose anal fin. Members have well developed sickle-shaped pharyngeal bones carrying 1-3 series of teeth. Seven genera are found in the lakes and rivers of Ethiopia (Shibru Tedlla, 1973).

2.2.4.1. *Carassius auratus* (goldfish)

This species is an introduced small to moderately –sized fish with a deep body and rounded cross section, large head and eyes with a small mouth and forked tail. Scales are large and the single dorsal fin has 3-4 stout spines at the lading edge. Color ranges from olive-bronze to deep gold along dorsal surface, fading to slivery-white along the belly (McDowall, 2000).

Buth *et al* (1991) have reported chromosome numbers of octaploid species in gold fish. Diploid chromosome number (2n) ranges from 100-162 (Klinkhardt *et al*., 1995).

2.3. Chromosome cytology

Karyotype is defined as the phenotypic appearance of the somatic chromosomes in contrast to their genetic content (Jackson, 1971; Fristrom and Spieth, 1980). The main components of karyotypes which can be observed for comparison of related species include absolute chromosome size, relative chromosome size, chromosome morphology and chromosome number (Sharma, 1991).

Differences in karyotype have aided many taxonomic decisions and have also provided telling clues in unraveling evolution (Stace, 2000). In spite of the accumulating molecular data, chromosome information continues to be important in assessing phylogenetic relationships (Carr *et al*., 1999), and also be of great importance in clarifying position of

a taxon (Takhtajan, 1997). Nowadays, a chromosome study has become a way of recognizing intra and inter- specific genetic variations etc. (Zima, 2000).

In fish species, chromosome numbers and/or chromosome arm numbers exhibit great variability, and one can assume that karyotype is specific (Lévéque, 1997). The number of chromosomes per cell, as well as the karyotypic configurations, varies from species to species but are characteristics within a species (Lévéque, 1997).

2.3.1 Reports on fish chromosomes

Of all known extant species of fishes, the chromosome numbers of only 650 to 700 species have been reported; complete karyotypes are known for only about 500 species i.e. 2-3% (Gold, 1979). For instance, according to Oliveira and Gosytonyi (2000) only 56 species among 646 species of the loricariidae family of catfish have been investigated at the cytogenetic level. Considering the number of species in this family, cytogenetic analysis are still scarce (Artoni and Bertollo, 2001).

The chromosome study of fishes has stimulated the interest of many researchers in the last few years. However, the small size and larger number of chromosomes in fish and the lack of a standard technique for fish chromosome preparation make their evaluation difficult (Thorgaard and Disney, 1990). Consequently the main difficulty in working with fish chromosomes is obtaining high quality metaphase spreads.

Another problem is that fish karyotypes are not identical, as in human beings or in other animal species. So we can not have a standard karyotype for fish because not only are there differences between species, but polymorphism often occurs within the same fish species (Al-Sabti, 1991). For instance, (Guegan *et al*.,1995) suggested that karyological data may be of use for clarification of African large *Barbus* and *Varicorhinus* although the comparative analysis of karyotype in this group is hampered by the high number of chromosomes of similar size, the inter and intraspecific variation found in the large barbel of Africa.

Chromosome number (2n) for the majority of cyprinid species is 2n=50 (Al-Sabti, 1991; Gul *et al.*, 2004), while *Cyprinus carpio* has 2n=98-100 and some *Barbus* species with 148-150 chromosomes have been reported as hexaploid from South Africa (Al-Sabti, 1991; Gul *et al.*, 2004); and from Ethiopia (Golubstov and Krysanov,1993). Berrebi (1998) has reported that the African 'large barbus' includes species with chromosome numbers of diploid (2n= 50), tetraploid (2n=100) and hexaploid (2n=150). Buth *et a*l. (1991) have reported that octaploid species of gold fish.

Many of the studied siluriformes have shown to possess 2n=54 or 2n=56 chromosomes with a fundamental number varying from 95 to 102 (Agnese *et al*., 1990). Comparative cytogenetic investigation of African catfish, *Clarias gariepinus* (Burchell), and Asian catfish *Clarias batrachus* (Linn) have diploid chromosome of numbers 56 and 50, respectively.

Cichlid cytogenetics has shown a conservative karyotype evolution with regard to diploid number. Most of the species possess 2n=48 chromosomes many of which are sub-telocentric chromosomes (Feldberg and Bertollo, 1985; Loureniro and Dias, 1999).

Table: 1.Chromosome number 2n and fundamental number (FN) of some freshwater fish species (Adapted from Lévéque, 1997).

Taxa	**Chromosome**	
	2n	FN
Family Cichlidae		
Oreochromis alealicus	48	
Oreochromis anderssoni	44	48
Oriochromis aureus	44	54
	44	44-50
	44	58
Oreochromis niloticus	44	62
Oreochromis mosambicus	44	62
	44	44-50
	44	
Tilapia zilli	44	54
	44	60

Tilapia rendalli	44	52
Family Claridae		
Clarias angularis	56	100
Clarias gariepinus	56	88
Family Mochokidae		
Synodontis schall	54	95
Synodontis sorex	54	96
Synodontis filamentosus	56	102

2.3.2 Variations in chromosome number

Basic chromosome number (x) is defined as the number of chromosomes in a set (Snustad *et al*., 1997). It is one of the most widely used characters in the study of biosystematics and there have been a vast amount of phylogenetic speculation using this value as a guide (Jones, 1970). Each species has its own specific base number.

Chromosome numbers and variability in chromosome numbers distinguishes certain major taxonomic groupings of fishes. For example, salmoniforme species have higher chromosome numbers than cypriniforme species, and they are more variable in chromosome number. These trends are observed at lower taxonomic levels. Groups with chromosome numbers in the range n=22-26 tend to be relatively invariant in chromosome numbers whereas groups with higher or lower chromosome numbers tend to be more variable (Gold, 1979).

Changes in chromosome number may occur in three ways. Sometimes, a chromosome number is increased to an exact multiple of the basic chromosome set. This mechanism is usually observed in a plant, which is referred to as polyploidy. The other process is Robertsonian rearrangements, where centric fusion of two non homologous acrocentric chromosomes produces a single metacentric or where centric fission of a single metacentric produces two non- homologous acrocentrics (telocentric) (Gold , 1979). The other one is where non-disjunction results in gain or loss of individual chromosomes, called aneuploidy.

2.3.3. Variation in genome size

Genome size, or the amount of DNA per nucleus, also shows wide variation among fishes. Bachmann *et al.*(1972), however, found that DNA content of 195 fish species, including representatives from all three classes, exhibited a normal distribution, skewed towards higher DNA values. Among the teleost fishes, the distribution is similar but the estimated mode (haploid amount) is 1.0 pg (Hinegardner, 1968, Hinegardner and Rosen, 1972). There is usually homogeneity of DNA amounts within families and lower taxonomic categories, and genome sizes tend to be relatively stable despite changes in morphology and /or physiology (Ohno, 1974).

Decreases in DNA content often are associated with increasing specialization in body form and design (Gold, 1979). More specialized species have less DNA per cell than do more generalized forms. This inverse relationship between genome size and degree of specialization holds for fishes as a group, and also within certain taxa (Hinegardner, 1968; Hinegardner and Rosen, 1972).

Within taxonomic families, a significant correlation exists between genome size and variation of genome size (Hinegardner and Rosen, 1972). Specialized families with small genome size tend to be less variable in genome size and almost all families with low average DNA contents per species (0.4-0.6 pg) have very little variation in DNA content among species.

One line of reasoning suggests that much of the reduction in genome size occurs during chromosomal rearrangements which produce change in chromosome number. Among diploid teleost fishes there is a highly significant, positive correlation between chromosome number and genome size (Hinegardner and Rosen, 1972).

One may further speculate that once a species reaches a small genome size, chromosomes structural changes which result in further DNA loss should no longer be easily tolerated. If this is true, highly specialized taxa should be relatively invariant in genome size and in karyotype. In the highly specialized order Perciformes, there is a relatively low genome

size when compared with most other teleostean orders. The Perciformes are also relatively homogeneous in genome size and chromosome number (Hinegardner and Rosen, 1972; Denton, 1973).

2.3.4. Variation in chromosome morphology

Karyotype of an organism is dynamic and it evolves in the course of time. The final goal of morphologic classification of chromosomes is to reach understanding of how this karyotype evolution has proceeded and to find correlation with the evolution of other systematic characters such as anatomical, biochemical and behavioral (Levan *et al*., 1964).

Chromosome morphology is studied at the metaphase of mitosis, when chromosomes become contracted to the maximum or nearly so and when they are stained (Sharma, 1991; Snustad *et al*., 1997). Chromosomes are arrested at mitotic metaphase, especially after treatment with spindle inhibiting chemicals, like colchicines, bromonaphtalene, colcemid, 8 hydroxyquinoline, and vinblastine (Jackson, 1971). Chromosomes have morphological features that may serve as landmarks. These include centromeric position, satellites, nucleolus organizer regions, and absolute and relative size of chromosomes.

2.3.4.1. Centromere

Centromere is a large constriction where the chromosomes appear to be pinched (Weaver and Hedrick, 1992). It is responsible for chromosomes alignment on metaphase plate and movement during mitosis and meiosis.

Chromosome morphology is studied on the basis of the primary constriction or centromere (Levan *et al*., 1964). The relative position of the centromere and thus the relative size of chromosome arms may vary between non homologous chromosomes, but it is constant for a given homologous pair. Therefore, the arm ratio is used for chromosome identification and classification (Sharma, 1991).

With respect to the relative position of the centromere, chromosomes can be categorized as metacentric (centromere at the middle), sub metacentric (centromere slightly of the midpoint), acrocentric (centromere near the end), and telocentric (centromere at the end). However, according to Leavan *et al.* (1964) chromosomes are classified based on the arm ratio (r=L/S, where r=arm ratio, L=long arm, S=short arm). Thus centromeric positions are assigned as median point (M), median region (m), submedian region(sm), subterminal region (st), terminal region (t), and terminal point (T) with corresponding arm ratio of 1.0,1-1.7, 1.7-3.0, 3.0-7.0, 7-∞ ,respectively.

2.3.4.2. Secondary constrictions and satellites

Some chromosomes in the complement contain a second constriction region other than the centromere, called secondary constriction between the primary and the end of chromosome arm (Weaver and Hedrick, 1992). This constriction is also known as nucleolus organizer regions (NORs).

2.3.4.2.1. Nucleolus organizer regions (NORs):- These are the origins of the nucleoli and are the major sites of rDNA genes clustered at specific chromosomal sites (Feneocchio, 2003). NORs can be localized directly with fluorescent in situ hybridization (FISH) with specific probes, or indirectly with the use of silver nitrate (Ag-NOR). The latter detects the transcriptional activity of the ribosomal genes during the preceding interphase (Hubel, 1985), since the silver binds to the nucleolar proteins and not directly to the r DNA (Miller *et al*, 1976). NORs can serve to complement the karyotypic variability analysis (Kavalco *et al*., 2005). In fish, the location of the 45S rDNA is an important cytogenetic marker, with some groups having only one pair of NORs, and others showing multiple NORs including one located on sex chromosomes (Bertollo and Cavallaro, 1992; Artoni and Bertollo, 2002).

2.3.4.2.2. Satellite

Satellite is a short piece of well stained chromosome distally that is pinched off due to secondary constriction. Although species differ in the number and size of satellites, diploid species have at least a pair of satellite chromosomes (Weaver and Hedrick, 1992).

2.3.4.3. Variation in chromosome size

2.3.4.3.1. Relative chromosome size

The relative chromosome size, as a component of karyotype, is therefore, used to characterize chromosomes. Chromosome complements of many species contain chromosomes of two contrasting sizes, large and small. Thus, karyotpe differences among species or taxa may be used to determine phenetic similarities and phylogenetic relationships. The chromosomal complements of most plant species consist of comparable size (symmetrical karyotype). However, there exists a graded series of chromosomal sizes (asymmetrical karyotype), as it is true for many animals including humans (Stebbins, 1971).

Degree of asymmetry in karyotype among fishes is broadly taxon specific. For example, in the trout (family Salmonidae), the karyotype is quite symmetrical. By contrast, the minnow (family Cyprinidae) is highly asymmetrical (Chen, 1969).

2.3.4.3.2. Absolute chromosome size

The absolute chromosome size including the total DNA content of the nucleus may vary between genera of the family having the same or similar basic chromosome numbers and even between species of the same genus (Stebbins, 1971; Sharma, 1991).

2.3.5 Chromosome banding pattern.

Structural banding techniques for chromosomes are of fundamental importance to cytogenetic and evolutionary studies. Since the introduction of banding techniques for mammalian chromosomes in the early 1970s, knowledge of mammalian evolutionary genetics, medical genetics and gene mapping has vastly expanded. In contrast, the application of these chromosomal banding techniques to poikilothermic vertebrates, including fish, seems very difficult. Most of the successful chromosome banding reports in published data on fishes are restricted either to C-bands or to replication bands (Hellmer *et al*., 1991). Distinct structural G-, R- and Q-banding patterns have only been described for the European eel *Anguilla anguilla* (Medranc *et al*., 1988). The difficulty in obtaining good-quality G-, R- and Q-banding patterns from the chromosomes of fish

seems to be related to their chromosome structure. All available data indicate that there is a strong relationship between genome compartmentalization by base composition and Q-, R- and G-banding on chromosomes (Schmid & Guttenbach, 1988). Unlike warm-blooded mammals and birds, whose genomes can be divided into GC-rich and AT-rich compartments, cold-blooded vertebrates including fish either lack or have little compartmentalization of their genomes by base composition (Medranc *et al*., 1988). These results indicate that, in order to obtain distinct multiple structural bands from the chromosomes of fish, new techniques, differing from the Q-, G- and R-banding of the chromosomes of warm-blooded vertebrates, should be used.

Obtaining multiple bands (G or R) along chromosomes is a difficult task in fish cytogenetics, and a few studies of Neotropical species using this approach have been reported. This was used, for instance, to characterize and investigate the origin of a multiple sex chromosome system (Bertollo *et al*., 1997), to compare different staining methods and banding techniques (Maistro *et al*., 1999), and to characterizing A and B chromosomes (Maistro *et al.,* 2000). Several multiple banding techniques (C-, G- and restriction enzyme banding) enable to allow the identification of several homologues in the chromosome complements.

2.4. The significance of cytogenetics and cytotaxonomy in systematics and evolution.

Cytogenetics refers to the study of heredity through the study of chromosome and the cytological mechanism of inheritance. Cytological characters, including chromosome number and karyotype analysis have been considered as reliable guides in studies of taxonomic and evolutionary relationships (Moore, 1968 and Stace, 1980). The karyotype can often contribute a great deal to the interpretation of phylogenetic relationships between different taxonomic groups (Marani and Falistocco, 1990). In spite of the accumulating molecular data, chromosome information continues to be important in assessing phylogenetic relationships (Carr *et al*., 1999).

The field of fish cytogenetics is still poorly developed. This is largely because of the difficulty in obtaining good-quality multiple structural banding patterns from the chromosomes of fish (Medranc *et al*., 1988; Hellmer *et al*., 1991).

Cytotaxonomy refers to the study of phenetic and /or phylogenetic relationships among species, based on comparisons of chromosome number and morphology. Chromosomal taxonomy can be quite useful, both in determining the phylogenetic relationships of the taxa, as well as in the segregation of siblings or cryptic species (Kapoor, 2001).

Chromosome studies have been proven themselves in time and again to be powerful tools in the resolution of taxonomic difficulties and in tracing evolutionary relationships (Smith, 1970).

In addition to morphological and molecular evidence, karyomorphology can be of great importance in clarifying the systematic position of a taxon (Takhtiajan, 1997). Animal cytogenetists have suggested that karyotypes might be important as isolating mechanisms in speciation and have their own evolutionary trends independent of genetic evolution (King, 1993).

Over the last three decades, cytogenetic studies in Neotropical fishes have widely contributed to the taxonomy of Neotropical fishes. It has been pointed out that

karyotypical analysis provides useful information for evolutionary and phylogenetic studies and aids in the identification of controversial species (Bertollo *et al*., 1986), and, indeed, such techniques have been successfully applied to characterize phenotypically similar cryptic species. Cytogenetical analysis of some fish species, e.g. from the genera *Leporirus* (Galetti *et al*., 1981) and *Brycon* (Margarido and Galetti, 1996) have agreed with their taxonomic status but cytogenetical studies of species such as *Astryranax scabrinnis* (Moreira-Filho and Bertollo, 1991), *Hopilas malabaricus* (Bertollo *et al*., 2000), *Eigenmannia virescens* (Almeida-Toledo *et al*., 2002) and *Hoplerythrinus unitaeniatus* (Diniz and Bertollo, 2003) have diversified from taxonomic classification suggesting that these group should be taxonomically reviewed.

3. OBJECTIVE OF THE STUDY

3.1 General objective

- To characterize the chromosomes of some fish species from Ethiopia rift valley lakes and some water bodies which can be useful for taxonomic and evolutionary status of the fishes.

3.2 Specific objective

- To document the chromosome number of the studied species.

4. MATERIALS AND METHODS

4.1. Specimen collection

4. 1. 1. Collection sites

The specimens in (Figure 1) for the present study were collected from four localities in Ethiopia. All the sites are found in the Oromia Regional State except Lake Chamo.

The selection of sites was based on the availability of lakes, fish diversity, and site accessibility. Specimen sizes, geographic locations of collection sites together with their longitude and latitude coordinates, altitude, species (genus) name as well as name of the lakes are presented in Table 2. The collection sites include:

1. Lake Chamo: - This Lake is situated in Arbaminch town which is about 505 Km to south of Addis Ababa in the Southern Nations, Nationalities, and People's Regional State. Fish specimens *Tilapia zilli, Oreochromis niloticus, Clarias gariepinus, Synodontis schall, Carassius auratus, Hydrocynus forsakii, Barbus bynni* were captured from the lakes.

2. Lake Ziway: - The lake is situated near Ziway town which is located 165 Km south of Addis Ababa. Specimens *Tilapia zilli* and *Oreochromis niloticus* were captured from the lake.

3. Lake Babogaya: - The lake is a crater lake, situated in Debre- Zeit town which is located at about 45 Km south East of Addis Ababa. Specimens *Tilapia zilli* and *Oreochromis niloticus* were captured from this lake.

4. **Sebeta artificial pond: -** This pond is found at about 25 Km to west of Addis Ababa, Specimens *Oreochromis niloticus* and *Carassius auratus* were captured from this pond.

Table 2. Specimen collection sites with their coordinates, altitude, specimen size, and species name.

Collection site	Coordinates	Altitude (meter)	Area (km^2)	Species name	Specimen code	Specimen number
Lake Chamo	$06^0$03'N, $37^0$36'E	1400	550	*Synodontis schall*	Cha-syn	5
				Clarias gariepinus	Cha-cla	6
				Oreochromis niloticus	Cha-oreo	2
				Hydrocyon forskaliii	Cha-hydro	1
				Barbus bynni	Cha-bar	3
Lake Ziway	$07^0$59'N $38^0$35'E	1636	442	*Oreochromis niloticus*	Zw-oreo	3
				Tilapia zilli	ZW-Zil	4
Lake Babogaya	$8^0$48'N $39^0$04'E	1600		*Oreochromis niloticus*	BG-Oreo	1
				Tilapia zilli	BG-Zil	6
Sebeta-artificial pond				*Oreochromis niloticus*	Seb-oreo	5
				Carassius auratus	Seb-goldfish	5

4.1. 2. Collection method

Specimens of fishes from the above mentioned lakes were collected by gill net. The samples were brought to the laboratory in plastic containers filled with the lake water. Photographs of the sample fishes were taken. (Figure 1)

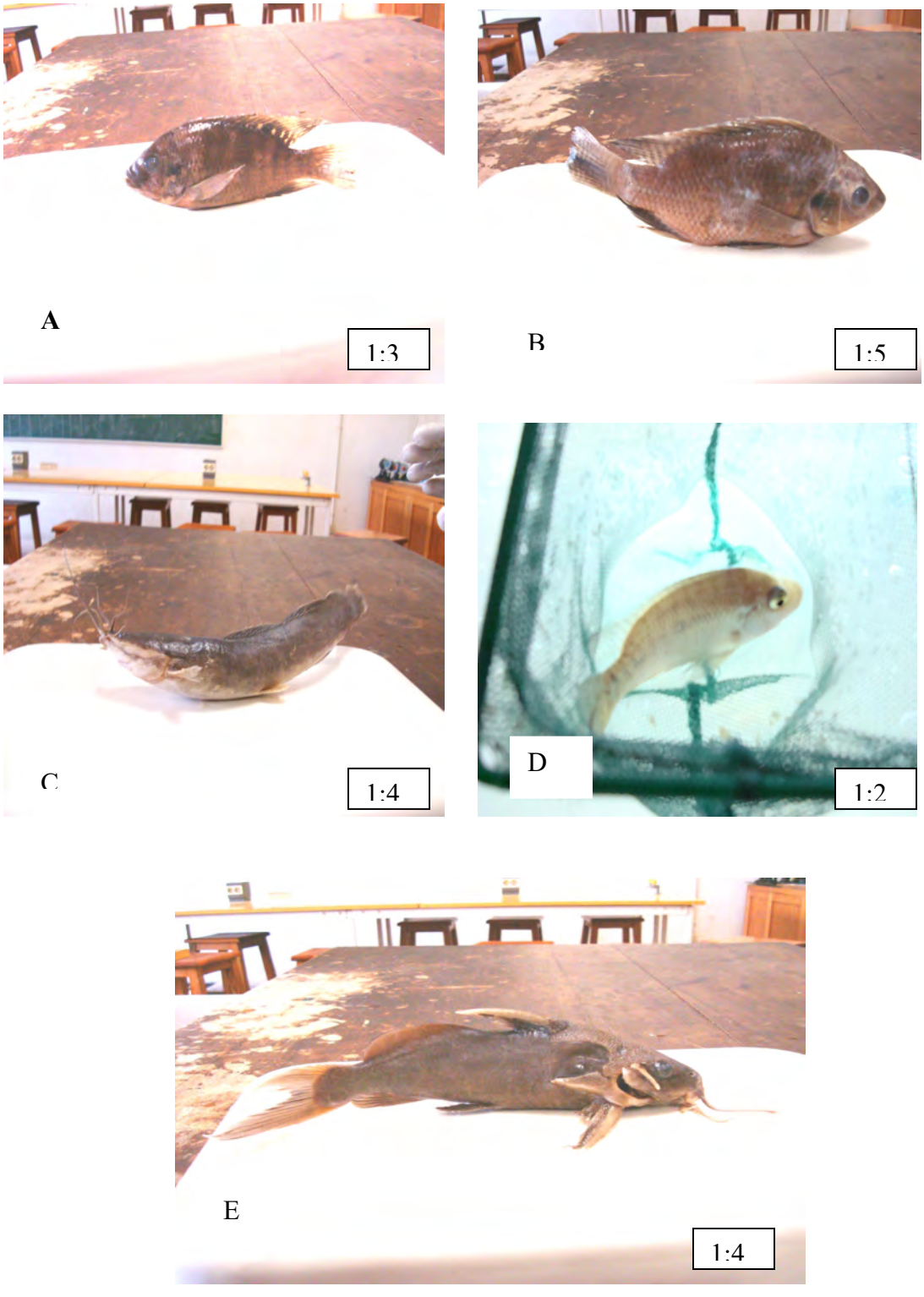

Fig1. Photographs of fishes used in the present study. A-*Tilapia zilli*, B- *Oreochromis niloticus,* C-*Clarias gariepinus,* D- *Carassius auratus and* E-*Synodontis schall*

4.2. **Specimens identification and preservation**

After morphological data were taken, the specimens were labeled and preserved in 4% diluted formalin and stored for taxonomic identification and later deposited in the Natural History Museum, Biology Department, for future reference.

Taxonomic identification was performed on preserved specimens. The specimens were identified to species level by Dr. Abebe Getahun.

4.3. Chromosome preparation

Colchicines injection was made in proportion to body size (0.5% of 0.8 ml /100gm body weight) and the fishes were placed in plastic containers filled with the lake water for about 6-8 hours for the chemical to arrest mitotic chromosomes at metaphase stage. Then, the gill filaments of the specimens were removed by forceps and kept in test tubes with a hypotonic solution (0.75M KCl). The tissues were kept for about 30 minutes in order to make cells swell with consequent dispersion of chromosomes within the cells. The gills were taken out of the hypotonic solution and were immersed into test tubes each containing about 10 ml of a fixative (3 methanol: 1 acetic acid) for 30 minutes.

Again, the gill filaments were taken out of the fixative and placed into a grinding glass for crushing with glass rod by adding a few drops of fixative. The cell solution filtered into a test tube using gauze. Then the filtrate was fixed by 1-1.5 ml fixative and allowed for centrifugation for 5 minutes (1000 rpm). Then, the supernatant was discarded and the pellet was fixed for further centrifugation. Finally the pellet was suspended in few drops of the fixative for slide preparation.

4.4. Slide preparation and staining

A few drops of the cell suspension were splashed on clean and dry slides using a pipette. The slides were then allowed to air dry and stored away until needed for staining.

The air dried slides were stained with Giemsa's stain (in phosphate buffer, pH 6.8) for 15-30 minutes or more until satisfactory staining was obtained. The slides were rinsed in distilled water, air dried and mounted under a 22x50 mm cover slip in DEPEX mounting medium.

4.5. Methods of Chromosome Analysis

Photomicrographs of metaphase plates with good chromosome spreads were taken with using a camera fitted microscope at a magnification of 1000 (i.e. 10x eyepiece and 100x objective). Chromosome number was determined from metaphase chromosome counts in the microscope as well as photographic prints. Count for each species were made from at least five to seven well-spread metaphase in intact cells.

5. RESULT

Chromosome analysis

Forty one fish specimens were captured during the fieldwork. The specimens represent four families and five species (Table 2).

Chromosome analysis has been made for five species. The chromosome analysis was not possible for the *Hydrocyon* and *Barbus* collected from Lake Chamo, which died before the sacrification time. Also that of *oreochromis* collected from Lake Chamo because of the lack of good metaphase spread.

For the five fish species, selected mitotic metaphase chromosome spreads are presented in Figure 2 to 5. The chromosomes of each species are characterized as follows.

5.1. *Tilapia zilli*

Specimens of this species were collected from Ziway and Babogaya Lakes. Metaphase spreads from the two specimens are present in Figure 2.A and B respectively. The diploid chromosome number obtained is 2n= 44

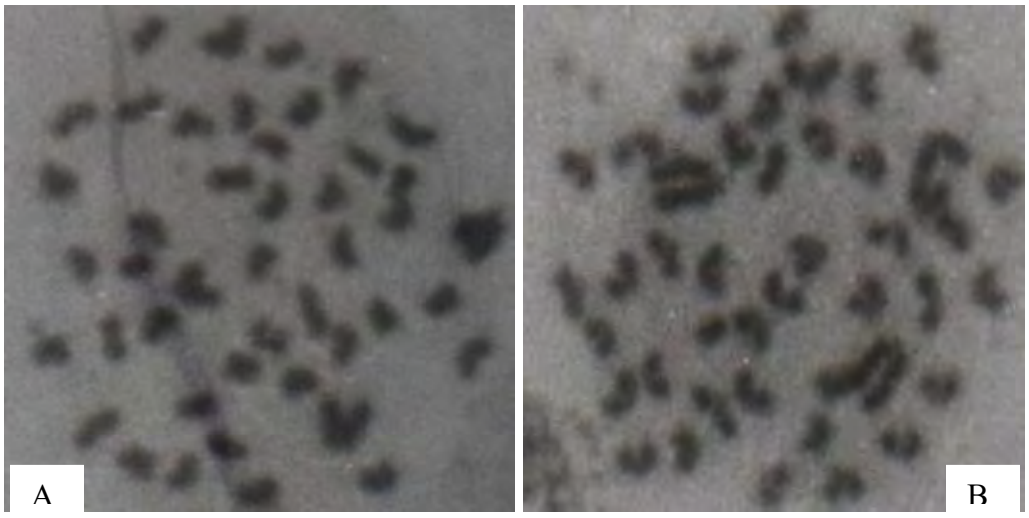

Fig.2.Metaphase chromosome spread of *Tilapia zilli*. A. specimens from Lake Ziway, B. specimens from Lake Babogaya. Magnification 1000 X

5.2 *Oreochromis niloticus*

Specimens of this species were collected from Lakes Ziway and Babogaya. Metaphase spreads from the three specimens are presented in Fig, 3A and B respectively. The diploid chromosome number obtained is 2n= 44

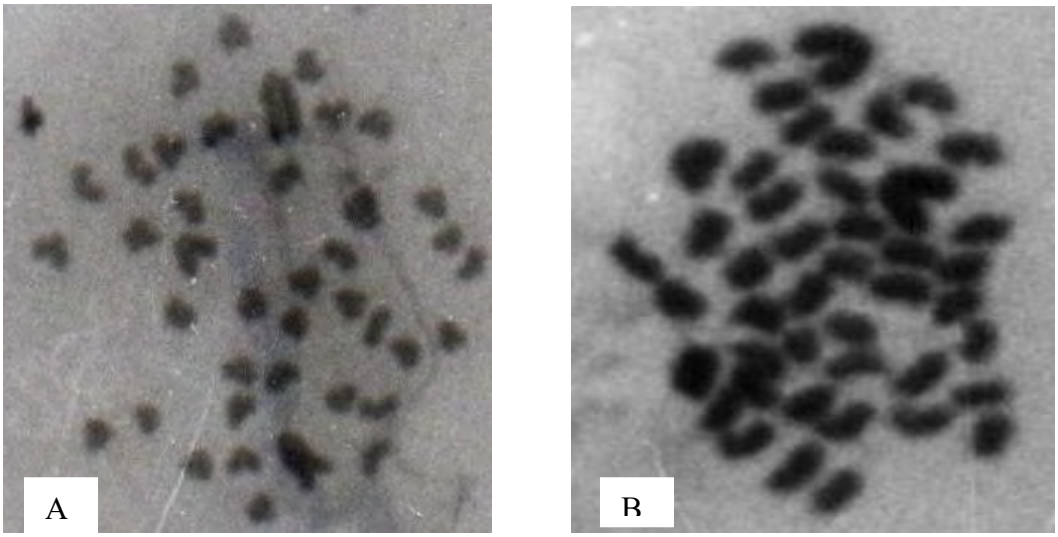

Fig.3. .Metaphase chromosome spread of *Oreochromis niloticus*.A. specimens from Lake Ziway,B. specimens from Lake Babogaya. Magnification 1000 X

5.3. *Clarias gariepinus*

Specimens of this species were collected from Lake Chamo. Metaphase spreads from the specimen is presented in Fig 4.The diploid chromosome number obtained is 2n= 56

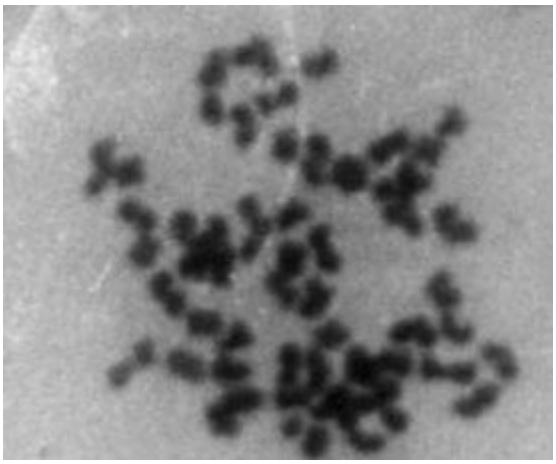

Fig.4. Metaphase plate of *Clarias gariepinus* specimen from Lake Chamo. Magnification 1000 X

5.4. *Synodontis schall*

Specimens of this species were collected from Lake Chamo. Metaphase spreads from the one specimen is presented in Fig 5. The diploid chromosome number obtained is 2n = 54

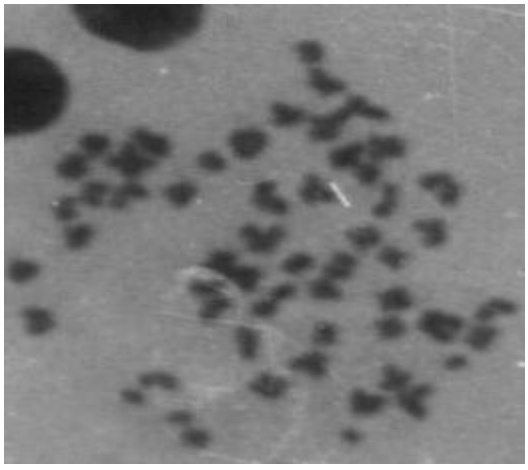

Fig.5. Metaphase chromosome spread of *Synodontis schall* from Lake Chamo. Magnification 1000 X

5.5. *Carassius auratus*

Specimens of this species were collected from Sebeta dam. Metaphase spread from this specimen is presented in Figure 6. The diploid chromosome number obtained is 2n=94

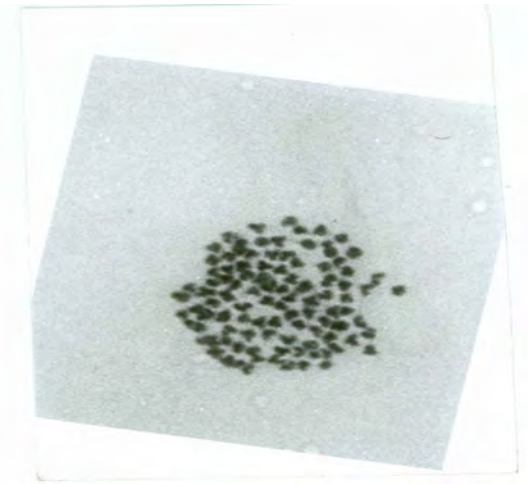

Fig.6. Metaphase chromosome of a specimen of *Carassius auratus* collected from Sebeta artificial pond. Magnification 1000 X

6. DISCUSSION

Due to the limitations rendered by the small size and large number of the chromosomes and lack of standard techniques for fish chromosome preparation it was not possible to give detailed cytogenetical characterization of all of the species. It has to be noted that some chromosomal morphological features are better observed under the microscope than using their photomicrographs.

Well-condensed and spread chromosomes usable for karyotypic analysis could not be obtained in all of the species under this study due to inherent and technical problems. Although it has not been possible to construct karyotypes for all of the studied species, chromosome counts have been made for all of the five species studied, namely *Tilapia zilli, Oreochromis niloticus, Clarias gariepinus ,Synodontis schall and Carassius auratus* Chromosome counts obtained for the first four species confirm the earlier reports made by Lévéque (1997).

In Ethiopia, chromosome study of fish is scanty. The only reports available are that by Golubstov (1996) and Berrebi (1998) on three species of *Barbus* in Lake Tana which include *B. intermidius, B. bynni and B. brevicephalus.*

Tilapia zilli, 2n=44

The chromosome count of *Tilapia zilli* species from Lakes Ziway and Babogaya revealed 2n = 44 (Fig. 2). This chromosome count agrees with that given by Lévéque (1997). Therefore, our count definitely confirms the earlier chromosome number reported for this species. However, this is the first description of chromosome for *Tilapia zilli* species from Ethiopia. Although this species collected from two different lakes, their chromosome number remains to be similar.

Oreochromis niloticus, 2n=44

The present finding of the diploid chromosome number (44) for *Oreochromis niloticus* from Lakes Ziway and Babogaya (Fig.3) is in agreement with previously reported by Lévéque (1997). This is the first description of chromosome for *Oreochromis niloticus* species from Ethiopia. Having the same diploid chromosome number by *Oreochromis niloticus* colected from different lakes, it prevails that environment has brought no effect in their chromosome number.

In our study the two ciclids *Tilapia zilli* and *Oreochromis niloticus* grouped under the order Perciformes both exhibit similar diploid chromosome number (44). These fish species are also morphologically similar and very difficult to distinguish except for small differences regarding their mean number of spines in the dorsal fin and and coloration. Despite the fact that the present study shows chromosome number similarity there might exist minor structural differences that would be revealed through molecular or high resolution cytogenetic techniques (Cuevas and Formas, 2003).

Clarias gariepinus, 2n=56

The somatic chromosome number obtained for this species (2n = 56) coincides with the prior determination (Lévéque, 1997) (Fig.4). Our present finding can be taken as a conformation on the earlier observation on the cytology of *Clarias gariepinus*. This is the first description of chromosome for *Clarias gariepinus* species from Ethiopia.

Synodontis schall, 2n=54

Lévéque (1997) indicated that *Synodontis schall* has 2n = 54 which is in agreement with our present report of 2n = 54 (Fig.5). Even though the first report of diplod chromosome number was described by Lévéque (1997), the present count is the first in Ethiopia.

Carassius auratus, 2n=94

The diploid chromosome number (94) described in this study for the species *Carassius auratus* from Sebeta artificial pond is the first description of chromosome from Ethiopia.

The present finding is different from a previous report on the chromosome number rang (2n = 100-162) of this species by Klinkhardt *et al*. (1995). Thus chromosome number (2n = 94) is presented here for the first time (Fig.6). Possibly this variation in chromosome number reduction may be due to aneuplody (Gold, 1979).

7. CONCLUSION

This study has reported for the first time, chromosome number of five species of fishes from Ethiopia and compared the results from other countries. Similar fish species that are collected from the different lakes exhibit similar diploid chromosome number.

8. RECOMMENDATIONS

The following recommendations are forwarded based on the results obtained.

1. Chromosome study has been found to be important for taxonomic and systematic study of fishes. It is, thus, recommended that more detailed cytological studies should be carried out on fishes of Ethiopia in order to better understand their taxonomy and systematics.
2. Further studies should be conducted thoroughly to investigate each rift valley lake as to the fish species inhabiting and encompass other water bodies and generate , biochemical and molecular data

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