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CHROMOSOME STUDIES OF FISH SPECIES FROM LAKE TANA, ETHIOPIA.

BY

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ABBREVIATIONS

FL= Fork Length

ST= Standard Length

TL= Total Length

LB.i= Large *Barbus intermedius*

LB.nedi= Large *Barbus nedgia*

LB.brevi= Large *Barbus brevicephalus*

SB.humi= Small *Barbus humilis*

SB.pleu= Small *Barbus pleurogrammma*

Ga.dem =*Garra dembecha*

Oreo.n,=*Oreochromis niloticus*

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ABSTRACT

Chromosome numbers are reported here for six species of three genera of Cyprinidae and one species of Cichlidae. Three of the species *Labeobarbus nedgia*, *Labeobarbus. brevicepalus* and *Labeobarbus intermedius* are endemic to Ethiopia and *Barbus humilis*, *Barbus pleurogramma, Garra dembecha* and *Oreochromis niloticus* are native to Ethiopia. Chromosome preparation were made after colchicine injection in proportion to body size (0.5% of 0.8 ml/100 gm) and the fish were placed in aquarium for about 6-8 hours to arrest mitotic chromosomes at metaphase stage. Then the specimens were sacrificed and the gill filament were removed and kept in hypertonic solution (0.75 M KCl), after about 30 minutes the gills were fixed (3:1 methanol- acetic acid). Part of them were preserved for the other time and part of them were taken to grinding dish for crushing with glass rods by adding few drops of fixative. The cell suspensions that were made by grinding of the gill filament were filtered by gauze and centrifuged for 5 minutes (1000 rpm) followed by splash slide preparation. Chromosomes were stained with Geimsa and mounted well. The following 2n chromosomes were observed for the studied seven species: *Labeobarbus nedgia,* 2n= 150*, Labeobarbus brevicephalus,* 2n=150*, Labeobarbus intermidus,* 2n=150*, B. humilis, 2n=50, B. pleurogramma,* 2n=50, *Garra dembeccha,* 2n=50 and *Oreochromis niloticus,* 2n=44. The chromosome numbers were determined from somatic mitotic chromosomes at metaphase stage. Polyploidy is evident in some of the species. Variable basic chromosome numbers were observed from the small and large *Barbus* fishes. Detailed studies of chromosome morphology were not possible because of the smaller size and large number of the chromosomes. Note on the taxonomy and distribution of each of the genera and species under study are provided. It was recommended that futher cytogenetical studies of the many species occurring in Lake Tana, Ethiopia are required for better understanding of the phylogenetic and evolutionary relationships of the species.

Key words: chromosome*, Labeobarbus*, *Barbus, Garra, Oreochromis,* Lake Tana, and phylogenetic

INTRODUCTION AND LITERATURE REVIEW

1 Fish and its diversity

Fishes are the most numerous constituting about 48.1% of all the major vertebrate groups (birds 20.7%, amphibians 6%, reptiles 14.4%, and mammals 10.8%) and also the major diverse group among the major vertebrate groups and accounts for more than half of all living vertebrates and are the most successful vertebrates in aquatic habitats world wide. There are about 25,000 living species of fishes, of which approximately 850 are cartilaginous, 85 are jawless and the remaining are bony fishes (Helfman *et al*., 1997). Fishes show a marvelous variety of morphological and behavioral adaptations.

Fishes are important as for commercial food, used as biological control agents, and are excellent subjects for the study and demonstration in biology teaching (Shibru Tedla and Fisseha H/ Meskel, 1981). The negative aspect of fishes is that throughout many orders of fishes there are species known to be poisonous to human (Bond, 1996).

The feeding habit of fishes is detritivores, herbivores, carnivores and ominivores. Within these categories fishes can be characterized further as 1) euryphagous, having mixed diet; 2) stenophagous, eating a limiting assortment of food types; and 3) monophagous, consuming only one sort of food. A majority of fishes; however, are euryphagous carnivores. Often the feeding mode and food types are associated with body form and digestive apparatus.

Over 97% of all known fishes are oviparous, that is, the eggs develop outside the mother's body. Examples of oviparous fishes include cyprinids, goldfish, and cichlids, in the majority of these species, fertilization takes place outside the mother's body, with the male and female fish shedding their gametes into the surrounding water.

According to Karl *et al.* (1977), fishes are found from Antarctic waters below freezing to hot spring of more than 40^0C and from freshwater to water saltier than the sea. Their vertical range of distribution exceeds that of any other vertebrate (Karl *et al.*, 1977).

Most (about 60%) of living fishes are marine, and the remaining live in freshwater; about 1% move between salt and freshwater as a normal part of their life cycle (Helfman *et al*., 1997).

According to Helfman (1997), geographically, the highest diversities are found in the tropics, the Indo-west Pacific area that includes the western Pacific and Indian Oceans and the Red Sea have the highest for a marine area, whereas south East Asia, South America, and Africa have the most diverse freshwater fishes (Golubtsov and Mina, 2003).

As eleswhere, ostariphysian fish dominates freshwater fish fauna of Africa but the families are unequally represented. Over 2000 non-cichlid species of freshwater fishes belonging to 340 genera and 75 families have been recorded in Africa according to the Check List of the Freshwater Fishes of Africa (CLOFFA) (Daget *et al*., 1991). Cyprinidae (Skelton *et al.*, 2001) Chracid and Siulriform families with Cyprinodontidae and Mormyridae are highly abundant in freshwaters. Many other families, including most of those that are endemic, are represented by only one or a few species. While the Cichlidae (Lowe –Mc Connell, 1991) are by far the most diverse with some 870 species and 143 genera recorded, most of them endemic to East African lakes (Daget *et al*., 1991).

The total number of freshwater fish species occurring in Ethiopian waters is substantially higher than those recorded from Sudan, Egypt, or Somalia (Golubstov and Mina, 2003). Fish species diversity and species composition vary greatly over the territory of Ethiopia. However, scientific data on Ethiopian fish fauna are limited because of access to many locations being very difficult (Golubtsov and Mina, 2003). The workers reported that the total number of valid fish species from Ethiopia fresh waters were between 168 and 183, while Abebe Getahun (2005b) reported about 153 valid species and subspecies of fishes belonging to 12 orders and 24 families and Daget *et al*. (1991) reported that fish fauna of

Ethiopia represents 12 orders, 25 families and 37 genera. The Ethiopian fish fauna can be categorized as Nilo-sudanic, East African and endemic forms (Roberts, 1975). There are about 10 exotic fish species introduced from abroad into Ethiopian freshwaters (Shibru Tedla and Fisseha H/Meskel, 1981). The number of endemic fish species of the country is estimated to range from 37 to 57 (Golubstovd an Mina, 2003).

The Baro-Akobo, Omo-Ghibe and Blue-Nile (Abay) basins contain representative of Nilo-Sudanic fish species. The Shebelle-Ghenale Systems and the Southern Rift Valley Lakes also harbor Nilo-Sudanic fish elements (Abebe Getahun and Stiassny, 1998). The system contains Nilotic fish species because the basins had /have past and present connections with the Nile and West and Central Africa river systems (Robrtes, 1975). Representative genera of Nilotic fish species in these water bodies are *Alestes, Bagrus, Citharinus, Hydrocynus, Hyperopisus, Labeo, Malapterurus, Mormyrus, Polypterus,* and *Protopterus* (Golubstov and Mina, 2003).

The highland East African fish forms, as recognized by Roberts (1975), are found in Northern Rift Valley Lakes of Ethiopia, the highland lake (Lake Tana) and associated systems and the Awash drainage basin (Abebe Getahun and Stiassny, 1998). Representative of highland East African fish species in these water bodies include the genera *Labeobarbus, Barbus, Clarias, Garra, Oreochromis,* and *Varicorhinus* (Abebe Getahun and Stiassny, 1998).

1.1 Fish diversity in lakes and river basins of Ethiopia

1.1.1 Ethiopian Rift Valley Lakes

The fish diversity and distribution of fish species recorded from Ethiopian Rift Valley Lakes have been reported by several authors (Getachew Tefera, 1993; Demeke Ademassu and Elias Dadebo, 1997; Abebe Getahun, 2001; Golubstov *et al.,* 2002; Golubstov and Mina, 2003). These studies have describe a total of 31 native and four introduced fish species from the Ethiopian Rift Valley Lakes and streams.

There are many isolated basins within Ethiopian Rift Valley, which make them distinct from the other regions of the country. While the expected correlation between the number of fish species and altitude is found in its drainages, the region does not follow the pattern. More so, the distribution of fish fauna is highest in its southern part, lowest in the central part and intermediate in northern part of the Rift Valley (Golubstov and Mina, 2003). They recorded 22-23 fish species from Abaya-Chamo-Chew Bahir system, 6-7 species of Awassa- Shalla system, 12 species from the Zwai–Langano-Abijata Shala system, 13-15 species for the Awash system and adjacent enclosed basins.

1.1.2 Lake Tana and other small lakes

The fish fauna of Lake Tana are included in the genera *Oreochromis, Clarias*, 'Large' barbs (*Labeobarbus*), 'Small' *Barbus*, *Garra, Varicorhinus* and *Nemacheilus* (Eshete Dejen, 2003).

There is one cichlid, *Oreochromis niloticus* (Nile -tilapia), which is the most widely spread Tilapia species in Africa and the catfish family (Claridae) is also represented by one species, *Clarias gariepinus* (African catfish), which is the most common member of its genus (Eshete Dejen, 2003). The largest fish family in the lake is the Cyprinidae, represented by the four genera, *Labeobarbus, Barbus, Garra,* and *Varicorhinus* (Eshete Dejen, 2003). Before 1990's, 'large' *Barbus* species of Lake Tana were considered as one large complex species, *Labeobarbus intermedius* (Tesfaye Wudneh, 1998). Later, after the work of many scientists (de Graaf, 2003; Eshete Dejen, 2003; Dimmick *et al.,* 2001; and Nagelkerke and Sibbings, 2000), the larger *Barbus* of Lake Tana where categorized into 15 morphotypes (species).

The 'small' *Barbus* of Lake Tana are represented by *Barbus pleurogramma* (Boulenger, 1902), *Barbus humilis* (Boulenger, 1902), *Barbus trispilopleura* (Daget, 1991) and *Barbus tanapelages* (de Graaf *et al*., 2000). However, Eshete Dejen (2003) hypothesized that *B. humilis* and *B*. *trispilopleura* are not separate species. The genus *Garra* is

represented in the Lake by *Garra dembecha* and *G. dembeensis* (Boulenger, 1911), *Garra tana* and *Garra regressus* (Abebe Getahun, 2000).

The genera *Varicorhinus* and *Nemacheilus*, are represented by the single species each, *V. beso* and *N. abyssincus*, respectively. Two exotic species, *Gambusia holbrooki* (Girrard, 1856), and *Esox lucius* (Linnaeus, 1758) were brought from Italy during the late 1930's and introduced into Lake Tana (Shibru Tedla and Fisseha H/ Meskel, 1981). However, there is no evidence that these species are still striving in the lake. Therefore, the total number of fish species of Lake Tana is 27 or 28, while fish species found in small lakes of Ethiopia is show in Table 1.

Table 1: Fish species in some small lake of Ethiopia (X represents absence of fish species; Source Genanaw Tesfay, 2006)

Lake	**Location**	**Fish species**
Hayk	North highland	*C. gariepinus O. niloticus*
Ashengie	North highland	*Common carp* (introduced)
Ardebo	North highland	*O. niloticus*
Zengena	North highland	Common carp (introduced) *Tilapia sp*.
Bishoftu Lakes	Near Debreziet town	*O. niloticus* *C. gariepinus* *A. antinori* *G. quadrimaculatus* *T. zillii* (introduced)
Metehara	Ethiopian Rift valley	*O. niloticus* *C. gariepinus*
Wonchi	Centeral highland	*O. niloticus* (introduced) *T. zillii* (introduced) *T. rendalii* (Introduced)
Dandii	Central highland	**x**

1.1.3 River basins

About 28-31 fish species belonging to 11 families and 19 genera were recorded from wabie Shebelle-Juba system within the limit of Ethiopian boundary (Golubstov and Mina, 2003; Abebe Getahun, 2005b) Table 2. Omo - Ghibe and their tributaries and Lake Turkana are home to 79 species of fishes, and of these species *Nemacheilus abyssinicus* is the endemic fish recorded from the basin (Golubstov and Mina, 2003) Table 2. The Baro - Akobo basin, the richest basin in its fish fauna is home to 91 species of fishes (Golubstov *et al*., 1995) Table 2. The number of fish species inhabiting the Abay (Blue - Nile) basin, excluding Lake Tana is about 45-46, of which three are endemic and one is introduced (Golubstov and Mina, 2003) Table 2. The number of fish species in Atbara - Tekeze basin is about 32, with 3 endemic and 1-2 introduced fish, belonging to 9 families and 22 genera (Golubstov and Mina, 2003).

Table 2; Fish species diversity in major drainage basins within the limit of Ethiopian boundary (Source: Golubstov and Mina, 2003)

River basin	Family	Genera	Indigenous	Endemic	Introduced	Total species
Wabi– Shebelle -Juba	11	19	28 -31	19	3	50-53
Omo– Turkana	19	42	79	51	0	130
Baro – Akobo	23	55	91	31	-	122
Atbara – Tekeze	9	22	32	3	1-2	36-37
Blue Nile	12	28	45-46	3	1	49-50

1.2 Cytology of fish

Chromosome number along with conventional morphology criteria data from paleontology, behavioral patterns, ecology and genetic experiments provide further tool for deciphering the phylogeny of fishes. The use of chromosome information are many but so far most of the work with fish has been concerned on the cytotaxonomy (Blaxhall, 1975). And the use of chromosome studies for determination of sex (in multiple sex chromosome mechanism) would be of value in fish breeding (Uyeno and Miller, 1971).

Cytogenetically every organism is characterized by its own specific karyotype both in number and morphology. However, recent reports indicate that a karyotypic variation may occur in different individuals of the same species, as well as between different species (Berrebi, 1998). It has been suggested by Roberts (1967) that fish are likely to have more intraspecific chromosome polymorphism than other vertebrates.

1.2.1 Chromosome Number

The number of chromosomes in cell as well as the karyotypic configuration varies from species to species, but it is a characteristic within species. In most fishes chromosomes occur in pairs (i.e. 2n or diploid), but some are polyploid. Chromosome numbers in fishes ranges from a low of n=8 in the cyprinidontid, *Notobranchius rachovii,* Gold, 1979) to a high of n=225 in the Cyprinidae, triploids derived from hexaploid level (about 450) polyploidy cyprinids (Berrebi and Rab, 1998). Chromosome numbers and variability in chromosome number distinguish certain major taxonomic grouping of fishes. It is important a datum for a species as many other characteristics considered significantly stable to merit taxonomic significance (Garber, 1992).

Table 3; Chromosome number list for some major Ethiopian freshwater fishes (Source, Leveque, 1997)

Species	**2n**	**FN**	**Author**
Protopterus			
Protopterus annectens	34	-	Wickbom, 1945 cited in Leveque, 1997
Protopterus dolloi	68	104	Vervoort, 1980a cited in Leveque, 1997
Polypteridae			
Calamaichthys calabarinus	36	**72**	Denton and Howell, 1973 cited in Leveque, 1997
Polypterus delhezi	36	**72**	Cataudella *et al*, 1978 cited in Leveque, 1997
Notopteridae			
Papyrocranns afer	34	38	Uyeno, 1973 cited in Leveque, 1997
Cyprinidae			
Barbus bynni	150	22	Golubstov & Krysanov, 1993
B. bynni occidentalis	148	-	Guegan *et al.*, 1995
B.aethiopicus	150	190	Golubstov & Krysanov, 1993
B.intermedius	150	216	Golubstov & Krysanov, 1993
B. ablabe	48	96	Rab *et al.,* 1995 cited in Leveque, 1997
B. viviparous	48	96	Post, 1965 cited in Leveque, 1997
Garra dembeensis	50	82	Golubstov & Krysanov, 1993
G.makiensis	50	84	Golubstov & Krysanov, 1993
G.quadrimacukata	50	88	Golubstov & Krysanov, 1993
Labeo sensogalensis	50	-	Pauty *et al*., 1990 cited in Leveque, 1997
Varicorhinus beso	150	86	Golubstov & Krysanov, 1993
Cyprinodontidae			
Aphysemian shti	36	-	Scheel, 1972 cited in Leveque, 1997
A.araoldi	38	**72**	Scheel, 1972 cited in Leveque, 1997
Northyo prancius rachovii	16	-	Ewulonu *et al*., 1985 cited in Leveque, 1997
Cichlidae			
Oreochromis alealicus	48	-	Park, 1974 cited in Leveque, 1997Jalabert *et al.,* 1974;
O. *niloticus*	44	62	Arai &Kolke, 1980 cited in Leveque, 1997

	44	64	Majumdar & Mc Andrew, 1986 cited in Leveque, 1997
Tilapia rendalli	44	52	Michele & Jakashi, 1977 cited in Leveque, 1997
T. Zillii	44	66	Majumdar & Mc Andrew, 1986 cited in Leveque, 1997
Claridae			
Clarias agularius	56	100	Agnese, 1989 cited in Leveque, 1997
Clarias gariepinus	56	88	Jeugel *et al*., 1992a cited in Leveque, 1997
Heterobrachcus longifilis	52	82	Jeugel *et al*., 1992a cited in Leveque, 1997
Bagridae			
Bagrus docmak	54	98	Agnese, 1989 cited in Leveque, 1997
Clarotes laticeps	70	102	Agnese, 1989 cited in Leveque, 1997
Mochokidae			
Synodontis schall	54	95	Agnese *et al*., 1990 cited in Leveque, 1997
S. sorex	54	96	Agnese *et al*., 1990 cited in Leveque, 1997
S.filamentosus	56	102	Agnese *et al*., 1990 cited in Leveque, 1997

1.2.1.1 Polyploidy

While polyploidy is a frequent phenomenon in plants (70% of angiosperms and up to 90% of pteridophytes) are considered polyploids (Solits and Soltis, 1999), it is argued that the polyploidy condition in animals is a disadvantage due to alterations in the distribution and the number of sex chromosomes and ensuring hormonal irregularities (Guegan *et al.,* 1995). However, they stated that some vertebrates, such as reptiles, amphibians and fish, perhaps due to their sex determination are known to be polyploid.

Polyplodization is another mechanism of speciation. Natural polyplodization is not rare event in fish, compared with other vertebrates. For example certain varities of *Carassius auratus* and large African *Barbus* species are known to be polyploid which have chromosome number of 148-150 (Oellermann and skelton, 1990). The majority of the cyprinid species have 2n=50 (Berrebi and Rab, 1998). Cyprinid fishes assigned to the genus *Barbus* constitute a polyphyletic assemblage so that they include at least three ploidy levels: diploid, tetraploid, and hexaploid. *Labeobarbus intermedius* , inhabiting Lake Tana (Ethiopia) is a hexaploid taxon (Berrebi and Rab, 1998). Recent karyological

studies (Golubtsov *et al*., 2002) have demonstrated that the small African *Barbus* are diploid (2n=50) whereas large African *Barbus* appears to be hexaploid (2n=150). That is the case for a few order cyprinids, including *G*. *dembeensis* and *Varicorhinus nelspruite* (Oellermann and skelton, 1990). Large *Barbus* in Ethiopia (*Labeobarbus bynni,. Labeobarbus intermidus, B. ethiopicus, Varicorhinus beso*) also have high number of chromosomes (2n=150) (Golubstov and Krysanov, 1993).

There are at least three cytological mechanisms which may bring about change in chromosome number: (1) Polyploidization, where the chromosome number is increased to an exact multiple of the basic chromosome set, (2) Robertsonian rearrangents, where centric fusion of two non-homologous acrocentric chromosomes produce a single metacentric, or centric dissociation of a single metacentric produces two non homologous acrocentrics (Robeston, 1916), and (3) aneuploidy, where nondisjunction or anaphase lag or endoreduplication results in gain or loss of individual chromosomes.

The advantage of polyploidy for fish is considered to be larger sized longer life, faster growth and greater ecological adaptability (Leveque, 1997).

1.2.2 Chromosome Morphology

Chromosome classification depends on size, morphology and centromeric position (Levan *et al.,* 1964). Based on chromosome size, chromosomes may arbitrarily be classified as long (>10 µm), medium (4.8 µm) or short (<2µm) (Levan *et al.,* 1964). However, measuring the length of chromosome from different cells of the same individual or different individuals of the same species may not give exactly the same figures, because the degree of contraction of chromosome varies depending partly on different conditions of chromosome treatments. Though measurements of chromosome length are sometimes inexact, they provide at least the best information on the relative size of the chromosome (Cohn, 1969). Fish chromosomes are smaller in size than chromosome in other vertebrates. According to Gold (1979), the length of the average fish chromosome is between 2 and 5µm, and many species possess numerous small

chromosomes of 2 µm or less, but which are nonetheless easily seen through light microscope. There are also very large chromosome of 15-30 µm in length, such as those found in the lungfish, *Lepidosiren paradoxo* (Gold, 1979).

Chromosome classification based on centromeric position, the centromere region usually appears to be constricted, and the position of constriction is most useful landmark, which is characterized by great consistency (Levan *et al*., 1964). Levan *et al*. (1964) recommended the definition of the relative position of the centromere on a chromosome arm ratio (r=L/S; L=long arm and S= short arm) in which chromosome classified at median point (M), median region(m), submedian region (sm), subterminal region (st), teminal region (t) and terminal point(T) with corresponding arm ratio respectively, of 1.0, 1.0-1.7, 1.7-3.0,3.0-7.0, 7.0-infinitiy and infinitive. However, the following alternatives are still terminologies in use to describe a chromosome. Telocentric: centeromere located at one end of the chromosome; acrocenteric, centromere located near one end of the chromosome so that it contains one long and one very short arm; submetacenteric, centromere located near one end of the chromosome than the other so that the two arms are distinctly unequal, but less than acrocenteric situations; metacentric: centromere located at or near the middle of the chromosome and its arms are nearly or quite equal in length. However, the distinction between these types is partly and highly dependent on chromosome size and quality. Some authors consider telocentric chromosomes as having just one arm, while other allocate two arms. This could explain differences observed for the same species and by different authors for the calculation of Fundamental Number (FN).

Discrimination between non-homologous chromosomes and pairing of chromosomes into homologous pairs is not easy, but now a days chromosome marking techniques have improved the accuracy of karyotyping. Cytological and molecular techniques produce a specific banding pattern on each chromosome that can be used for comparative analysis (Oziut-Costaz and Foresti, 1992). Until 1997, only a few studies have adapted the chromosome banding method to study the chromosomes of lower vertebrates such as fish (leveque, 1997).

In fish, chromosome banding is primarily limited to visualization of the chromosomal nucleolus Organizer Regions (NORs), which is chromosomal site responsible for the synthesis of ribosomal RNA. The silver staining of NORs is a simple means of localizing the gene coding for 18s and 28s ribosomal RNA in chromosome. This method is not very powerful compared to the banding technique, but much easier to apply. In fish, different studies have revealed only NOR-bearing chromosome pair for the species studied, except for several cyprinids and few other species (leveque, 1997). Those chromosomes and their position of NORs usually differ between species and could be used for their differentiation.

C- banding is another technique that has been successfully applied to fish. It allows one to visualize the constituive of heterochromatin corresponding to the highly repeated DNA sequences which are in the centromere region and sometimes close to the NOR location in fish chromosome; C- banding may be effective to identifying homologous chromosomes and thus characterize and differentiate species (Oziut-Costaz and Foresti, 1992).

Microsatellites are a useful way to estimate heterozygosity of stocks, and will be very useful for tracking parentage in selection experiment. For example, based on microsatellite, it will be useful for classifying tilapia strains to species, or to identifying the probable hybrid origins (Kocher *et al*., 1997).

1.2.3 Speciation

The small, *B. tanapelagius* has recently evolved from small *B. humilis* (de Graaf, 2003). The genetic diversity of the mtDNA cytochrome b gene among Lake Tana's large *Barbus* species is very low. The failure to distinguish between species is possibly due to the recent origin of the species flock. The 15 large, hexaploid (Golubstov and Krysanov, 1993) endemic *Barbus* species (Nagelkerke *et al.,* 1994; Nagelkerke and Sibbing, 2000) have exploited their potential for trophic diversification to the fullest, including the cyprinid unexpected speciation for piscivory (Sibbing and Nagelkerke, 2001). According

to Nagl *et al*. (2000) the major lineage divergence took place about 100,000 years ago, but the vast radiation of ecomophological diversity within Lake Victoria's haplochromine species flock occurred in the last 15,000 years (Johnson *et al*., 1996). The age and origin of the assumed monophyletic (Nagelkerke and Sibbing, 1998) *Barbus* species of Lake Tana is at present unknown.

According to Tsigenopoulos and Berrebi (2000) all large *Barbus* species from Lake tana cluster together with Ethiopian *Barbus*, showed very little divergence. *Barbus intermedius* from Lake Chamo formed a distinict group from the large barbs from Lake Tana and Dedidessa River. *B.humilis* and *B. tanaplegius* cytochrome b sequences cluster together and show very little sequence divergence. *Barbus pleurogramma* from Lake Tana clusters together with *B. paludinosis* from other lakes and rivers in Ethiopia.

1.3 General description of the fish under study

1.3.1 Family *Cichlidae*

The family Cichlidae in the order of Percomorphi suborder Percoidea in the class of Neopterygi, has well described genera (*Astatotilapia, Aulonocara, Hemichromis, Oreochromis, sarotherodon, Sersanochromis, Tilapia and Danaklia*) from the genera *Hemichromis, Oreochromis, Tilapia* and endemic *Danakila* are found in Ethiopia. The family is widely distributed in freshwaters of Africa, Central and Southern America and in Israel (Shibru Tedla, 1973). *Oreochromis* is the only genus found in Lake Tana which has only one species O*reochromis niloticus.*

1.3.1.1 *Oreochromis niloticus,* Linnaeus,1757

Description: Head naked, snout rounded protuberance absent on the dorsal surface of the snout. Mouth moderately large, outer jaw contains several teeth in 4 series rows. Eye visible on the dorsal only (supero- lateral). Body somewhat compressed laterally. Caudal peduncle depth has equal length, scale cycloid. Lateral line interrupted. Dorsal with xII 11- xvIII 12: anal fin III8-III9. Caudal fin truncated with 8 – 10 faint vertical bars.

Coloration: Dark grayish color above, white below.

Distribution: The species is widely distributed in almost all lakes and rivers of Ethiopia (Shibru Tedla, 1973).

Importance: O. niloticus is important for ecological balance, commercial food, and aquaculture. The total production of tilapia in the year 1997 exceds 659,000 metric tone per year, (FAO Fisheries Statistics, 1997 cited in Kocher *et al*., 1998) of which the majority consists of the species *O. niloticus*, which is the first commercially important fish species in Ethiopia (Tesfaye Wudneh, 1998).

Cytology: The chromosome number of *O. niloticus* is 2n=44 (Kocher *et al.,* 1998). The karyotype of various tilapia species are highly similar, consisting of 22 pairs with no morphologically distinct sex chromosomes (Kocher *et al*., 1998). In fact, only two pairs are recognizable; the remaining 20 being smaller in size and morphology. At molecular level, the genome size of the several species had been measured at around 1 pg (1000 Mb), about one third of many mammalian genomes. The genome varies by up to 44% among species from 0.84 to 1.21 pg (Kocher *et al*., 1998).

1.3.2 Family *Cyprinidae*

The family Cyprinidae is very rich in species number (>2000 species; Nelson, 1994) and widely spread of all freshwater fish families and even the largest family among all vertebrates (Leveque *et al*., 1997; Skelton *et al*., 1991). Its classification is in the suborder of cypinoidea and in the order of ostariohpysi under the class of Neopterygii

(Shibru Tedla, 1973). This family comprises several genera (*Barbus, Garra, Varicorhinus, Barilius, Engaulicypris, Chadaethops and Nemacheilus*) and several species that are widely distributed.

The largest genus, *Barbus*, (>800 species) is a polyphyletic assemblage, including, diploid, tetraploid and hexaploid species ranging in size from a few centimeter to over a meter (Howes, 1991). Numerous African species assigned to the genus *Barbus* belong to two distinct groups, 'Small' *Barbus* (about 230 species) and 'large' *Barbus* (about 70 species). These two groups differ especially in size (i.e. about 150 mm SL and 700-900 mm SL) respectively and type of scale serration (radiating Vs longitudinal, respectively) (Golubstov and Krysanov, 1993). The small *Barbus* species can easily be separated from juvenile large *Barbus* species by a weakly developed first dorsal spine, and radiating straite on the scale (de Graf *et al*., 2000). Hexaploid barbs*:* The lineage of African hexaploid barbs is clearly monophyletic. This homogeneous group is widespread in Africa and treated as a single genus. Golubtsov and Krysanov (1993) and Berrebi _(1998) proposed erecting the name of the subgenus to which they are classified (*Labeobarbus*) to generic rank (Doadrio, 1994; Berrebi, 1995).

The Cyprinidae in Lake Tana, Ethiopia, belong to four genera, i.e. *Barbus, Varicorhinus* and *Garra*. The genus *Barbus* of Lake Tana, based on its size is classified into 'large' and small *Barbus* and the former includes 15 species (*Labeobarbus tsanesis*, *Labeobarbus brevicephalus, Labeobarbus intermedius*, *Labeobarbus truttiforms*, *Labeobarbus macrophtalmus*, *Labeobarbus megastoma, Labeobarbus acutirotris, Labeobarbus palatydorsus, Labeobarbus gorgorensis, Labeobarbus crassibarbis, Labeobarbus dainellii, Labeobarbus gorguari, Labeobarbus surkis, Labeobarbus nedgia, and Labeobarbus longissimus*) whereas the 'small' *Barbus* include (*B. humilis, B. pleurogramma*, and *B. tanapelagius*) (Eshete Dejene, 2003). The genus *Varicorhinus* contains only a single species *V. beso*. The genus *Garrar* is represented by *G. dembecha*, *G. dembeensis* and the two endemic species, *G. regressus*, and *G. tana* (Abebe Getahun, 2007; de Graaf, 2000). Of the cyprinid, a genus Barbus is by far the most diverse group and is found in many water bodies investigated. The group stands second next to *O.*

niloticus, in commercial catches from the countries water bodies (Abebe Getahun and Stiassny, 1998).

1.3.2.1.1 *Labeobarbus nedgia* (Lip), Ruppell,1836

Description: Mouth inferior, protractile with a large upper jaw extension, lower with a well developed median lobe; jaws rounded. Very short 2 parts circum oral barbells present. Eyes are relatively small, head length is a little less than body depth. It has a relatively short snout. Body covered with cycloid scales. Dorsal equally distant from the lip of snout and the caudal, scales longitudinally straitened. Caudal fin forked. The size ranges from 103 to 707 mm FL.

Coloration: Variable in color, from light yellowish brown with a silvery sheen to golden brown, olive green and dark brown. Back is always darker than the sides. Fins are variable in color, whitish, some times, a red tinge but darker brown.

Distribution and ecology: It is endemic to Lake Tana (Nagelkerke, 1997). However, it is found in River Angreb and Sanja (Genanaw Tesfaye, 2006). *Labeobarbus nedgia* occurs especially over rocky substrate in water of more than 6 m depth. The diet consists of mainly macro benthivorous. It is the only *Barbus* species in Lake Tana that eat water crabs (Bond, 1996).

*Importance: S*ource of food.

1.3.2.1.2 *Labeobarbus brevicephalus* (Short head),

Description: The head length is shorter than the body depth: upper and lower lips thin, inferior mouth with protrusion, lower jaw does not extend anterior of the upper jaw, anterior barble less than two times in the gape width. Head length is more or less 1.3 times in body depth: head length is more than 4.2 in SL: head width more than 1.8 times in head length. Body depth is less than 3.6 times in SL, the eye diameter is more or less 1.6 times interorbital width. The size ranges from 89 to 317 mm FL.

Coloration: Light yellow green some times with bluish tinge on slightly darker back. Fins are greenish especially the pectoral fin is often tinged orange or reddish.

Distribution and ecology: Occurs over muddy, sandy and rocky substrate all over the lake, preferable in less than 6m depth, mainly feed on zooplankton.

Importance: Source of food.

1.3.2.1.3 *Labeobarbus intermedius,* Ruppell,1837

Description: Depth of body contained 3 1/5 to 4 times in TL, length of a head contained 3 ¾ to 4 1/2 times in TL. Snout rounded 3 to 3 ½ times in length of a head; eye contained 3 ½ to 6 times in length of head; interorbital width contained 2 2/3 to 3 ¼ times in length of head; mouth inferior, its width 4 to 5 times in length of a head; lips moderate, two barbels on each side. Dorsal fin v 8 – 9, a little nearer occiput than caudal fin, boarder concave. Anal fin III5 often reaching caudal fin. Pectoral as long as or a little shorter than head. Caudle peuncle 1 ½ to 2 as long as deep. Scales longitudinally striated, 30 – 38, 2 ½ to 3 between lateral line and pelvic fin, 12 (rarely 14) round caudal peduncle. The size ranges from 120 to 400 mm.

Coloration: Olive or green above, yellow or pinkish beneath; fins brown or olive, caudal some times green.

Distribution and ecology: It is widely distributed in freshwater of Ethiopia (Genanaw Tesfaye, 2006). It is commonly found on the shore area of the lake and it is macrophytivorous (de Graaf *et al.,* 2003).

Importance: Source of food.

Cytology: The reported chromosome number with its fundamental number is 2n=150, FN=216 (Golubstov & Krysanov, 1993). Finding of Ollerman and Skelton (1990),

Golubtsov and Krysanov (1993), and Gue'gan *et al*, (1995) have shown that the African large *Barbus* are hexaploids (2n= 150), compose a well defined and probably monophyletic group. Although they suggest that comparative analysis of karyotype in this group is hampered by the high number of chromosomes and similar size, the inter- and intraspecific variation found in the African *Barbus*, may be used for clarification of their phylogeny and taxonomy.

1.3.2.1.4 *Barbus humilis,* Boulenger, 1902

Description: Depth of the body contained 3 1/3 to 3 ¾ times in TL, length of the head contained 3 2/3 to 4 times in TL. Snout rounded, shorter than eye, which is contained 3 to 3 ½ times in length of head; interorbital width contained 1 ½ to 2 2/3 times in length of head; mouth small terminal; lips feebly developed; two barbells on each side. Dorsal fin III8 equally distant from anterior border of the eye and from caudal fin, border feebly concave; last simple ray is not enlarged , not serrated, nearly as long as the head. Anal fin III5, not reaching caudal fin. Pectoral fin 2/3 to ¾ length of head, not reaching pelvic fin base of latter below anterior ray of dorsal fine. Caudal peduncle nearly twice as long as deep. Scales radiately straited, 29 -31, 2 between lateral line and pelvic fin 12 -14 round caudal peduncle. The diagnostic character for identification in the field is with presence of three dark lateral lines, no spots (Eshete Dejen *et al*., 2002). Its body size is about 56 mm.

Coloration: Silvery, brownish in the back; an indestinict lateral bands; fin whitish.

Distribution and ecology: *B. humilis* is abundant in shallow area (0 – 4m depth), over rocky, sandy/muddy substrate and decrease sharply in abundance with increasing depth from the shore. By its number it has a large biomass and is important prey item for the larger piscivorous *Labeobarbus* species (Eshete Dejen, 2003).

Importance: At present small *Barbus* are not being fished, but they are good source of protein is under investigation (Eshete Dejen, 2003)

1.3.2.1.5 *Barbus pleurogramma,* Boulenger, 1902

Description: Depth of body equal to length of head contained 4 times in total length, snout rounded, shorter than eye, which contained 31/2 times in length of head; mouth terminal, its width contained 4 ½ times in length of head; lips feebly developed; two barbells on each side. Dorsal fin III 7, equally distant from center of the eye and from root of the caudal fin, border straight; last simple ray bony, strongly serrated behind, 4/5 length of head. Anal fine III 5, not reaching caudal fin. Pectoral fin 2/3 length of head, not reaching pelvic fin. Caudale peduncle twice as long as deep. Scales radiately straited, 35, 3 between lateral line series and pelvic fin 16 round caudal peduncle. Total length of the *B. pleurogramma* is about 400 mm.

Coloration: Silvery, brownish on the back; a black line along each side of the body.

Distribution and ecology: Found in Lake Tana and rivers flowing into the lake. *B. pleurogramma* lives in along the submerged vegetation in the flood plains.

Importance: At present small *Barbus* are not being fished, but they are good source of protein is under investigation (Eshete Dejen, 2003)

Cytology: Oellermann and skelton (1990), reported the chromosome number of African small *Barbus* as diploid 2n=50, but there is no karyotype report on these two species (*B. humilies and B. pleurogramma*).

1.3.2.2 *Garra dembecha,* Boluenger, 1911

Description: Body feebly compressed, its depth contained 3 2/3 to 6½ times as total length. Head moderately depressed its length contained 4 ¼ to 5 times in total length; snout rounded. Eye in the second half of the head. Lips covered with granular papillae, the upper will developed, 2 small barbells on each side. Dorsal fin with 7 rays, anal fin with 5 rays. Caudal peduncle 1 1/3 to 1 ½ times as long as deep. Lateral line with 36 to 40 scales. The total length of *Garra dembecha* is about 160 mm.

Coloration: Olive green or brown above, yellowish beneath; a serious of black spots on the dorsal fin near to its base.

Distribution and ecology: The genus *Garra* is distributed throughout Asia and Africa, but 60% of African species are found in Ethiopia (Abebe Getahun and Stiassy, 1998). G*arra dembecha is* one of these species and is found in Lake Tana and many rivers in Ethiopia

Importance: Source of food (as I know).

Cytology: There is no chromosome number report on this species.

2. The present study

The study of karyotype in fish has stimulated the interest of many researchers in the last few years (Demirok and Onlo, 2001). However, small size and larger number of chromosomes in fish, and the lack of standard technique for fish chromosome preparation, makes their evaluation difficult (Demirok and Onlo, 2001). Cytological studies, including chromosome number and karyotype analysis have been considered as a reliable guide in the study of taxonomic, evolutionary studies, variation within and between populations, genetic control in fish breeding, etc (Ozout-Costaz and foresti, 1992).

In fish, chromosome numbers ranges from 2n=16 to around 2n=450. According to leveque (1997) the kayotype of many African species is not well known despite the existence of a great deal of data on chromosome numbers. But, in view of fundamental significance of chromosome number in systematics and evolution, chromosome number data of fish is incomplete.

Reports on chromosome number and kayotypes of the fish family Cyprinidae and Cichlidae are not complete. The chromosome number of many species of the genera

Barbus, Garra, and Oreochromis found in Ethiopia has not been totally reported. Thus, the following study was conducted to fill this gap.

2.1 General Objectives of the Study

- The main objective of the study is to generate chromosomal information for some fish species of Cyprinidae and Cichlidae occurring in Lake Tana, Ethiopia.

2.2 Specific Objective of the Study

The specific objectives of this study are:

- To determine chromosome number of the species in the genera *Labeobarbus*, *Barbus*, and *Garra* in the family Cyprinidae and *Oreochromis* in the family Cichlidae.

3 Study Area

Lake Tana

Lake Tana is situated in the North western highland of Ethiopia, 565 km north of Addis Ababa, (Fig.1) at an altitude of 1830 m. It is turbid, oligo-mesohrophic, shallow lake (average depth 8 m, maximum depth 14 m) covers an area of 3200 km^2 and is Ethiopian's largest lake, containing half of the countries freshwater (Eshete Dejen, 2003). Several rivers feed into the lake. Lake Tana is the souce of the Blue-Nile, which is the only river flowing out of the lake. High waterfalls (40m) at Tissisat, 30 km downstream from the Blue Nile outflow, effectively isolate the lake's icthyofauna from the lower Nile basin. Three fish families occur in Lake Tana. The Cichlidae and Claridae are represented by only one species each, *Oreochromis niloticus*, and *Clarias gariepinus,* respectively. The largest fish family in the lake is Cyprinidae, which is represented by four genera, *Varicorhinus, Garra*, *Labeobarbus* and *Barbus* species. *Varicorhinus* is represented by a single species *V. beso*. According to Boulenger (1911), the genus *Garra* is represented by two species in Lake Tana, *G.dembecha* and *G.dembneesis* but the description are inconsistent (Nagelkerke, 1997). However, in Lake Tana *Garra* might form a mini-species flock with at least two endemic species "*G. regressus"* and *" G. tana"* and may be even more (Abebe Getahun, 2000). The genus *Barbus* has been revised several times. The latest revision of the large hexaploid barbs of Lake Tana (Nagelkerke and Sibbing, 2000), distinguished 15 different species. Three small diploid *Barbus* species are present in Lake Tana: *B. humilis* (including the previous *B. trispilopleura*, Eshete Dejen *et al*., 2002), *B. pleurogramma* and the recent discovered *B. tanapelagius* (de Graaf *et al*., 2000).

Rain fall, water level, water temperature and vertical transparency all follow seasonal patterns (de Graaf *et al.,* 2003). Rain fall peak is in July-August, followed by a rise in the level of the lake, peaking in September-October. During the rainy season the vertical transparency is reduced due to inflow of large amount of silt, resulting from severe erosion by the affluent rivers. There is annual variation in water temperature 20-24 ^{0}C (de Graaf *et al.,* 2003).

Even though, it is less than that of other lakes in Ethiopia commercial production of fish per year is around 820 tones (Tesfaye Wudneh, 1998)

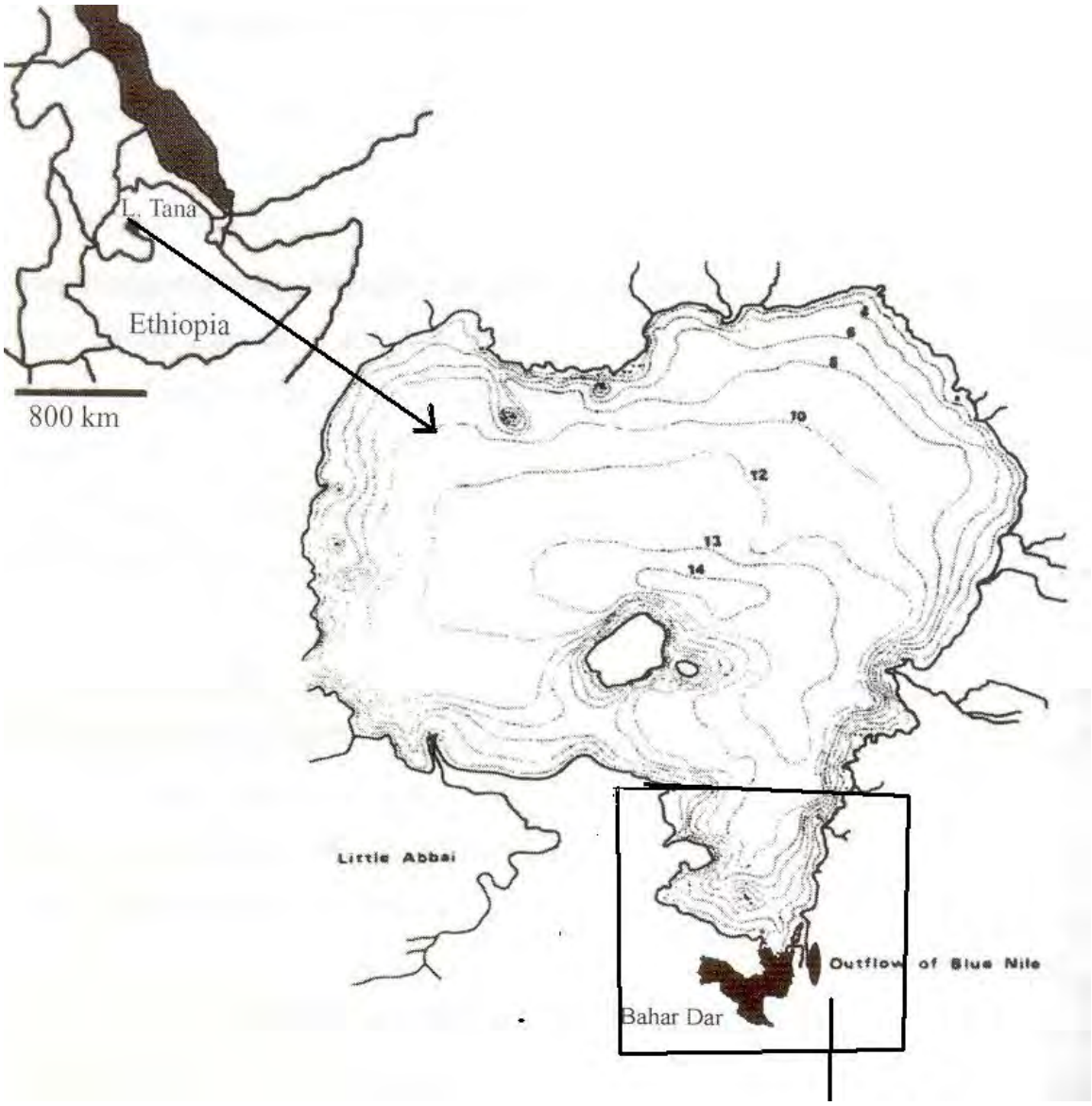


Fig 1 Map of Lake Tana (source; Eshete Dejen, 2003)

4 MATERIALS AND METHODS

4.1. Specimen collection

4. 1. 1. Collection site

The specimens for the present study were collected from Lake Tana, Ethiopia (Fig.1). The site is found in the Amhara Regional State: The selection of site was based on the availability of fish diversity, and site accessibility.

4.1. 2. Collection method

Specimens of young fishes were collected from Lake Tana by gill net Table 4 and Fig. 2-4. The samples were brought to the laboratory.

Table 4 List of fish species collected in Lake Tana

Fish species	Number of fish collected
Cyprinidae	
Labeobarbus intermedius	15
Labeobarbus nedgia	7
Labeobarbus brevicephalus	13
Barbus humilis	17
B. pleurogramma	16
Garra dembecha	13
Cichlidae	
Oreochromis niloticus	16

(A) *Labeobarbus nedgia* **Scale 3:1**

(B) *Labeobarbus intermedius* Scale 1.5:1

(A) *Labeobarbus brevicephalus* Scale 1.5:1

Fig.3: Photograph of fish species: (A) *Labeobarbus nedgia* (B) *Labeobarbus intermedius,* and (C) *Labeobarbus brevicephalus*

(A) *Barbus humilies* Scale 2:1

Barbus pleurogramma Scale 2:1

(B) *Garra dembecha* Scale 1.5:1

(C) *Oreochromis niloticus* Scale 2:1

Fig.4: Photograph of fish species: (A) *Barbus humilies* (B) *Barbus pleurogramma,* C) *Garra dembecha* and (D) *Oreochromis niloticus*

4.2. Specimen identification and preservation

After morphological data were taken, the specimens were labeled and preserved in (9 water: 1 formalin) and stored for taxonomic identification and later retained in the Natural History Museum, Biology Department, for future reference.

Taxonomic identification was performed on preserved specimens. The specimens were identified to species level with the kind collaboration of Dr. Abebe Getahun Addis Ababa University, Ethiopia.

4.3. Chromosome preparation

Colchicine injection was made in proportion to body size (0.5% of 0.8 ml /100gm) and the fishes were placed in aquarium for about 5-8 hours for the chemical to arrest of mitotic chromosomes at metaphase stage. Then, the specimens were sacrificed and the gill filaments were removed and kept in hypotonic solution (0.75 M KCl). The tissues were kept for about 30 minutes in order to make the 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 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 suspension was filtered into a centrifuge tube using cloth 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 about 1 ml 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 from a height of about 1 meter. The slides were then allowed to airdry 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 X10 occular lens and X1000 objective lens with total magnification of X1000 using a camera fitted microscope. The 2n was determined from metaphase chromosome counts.

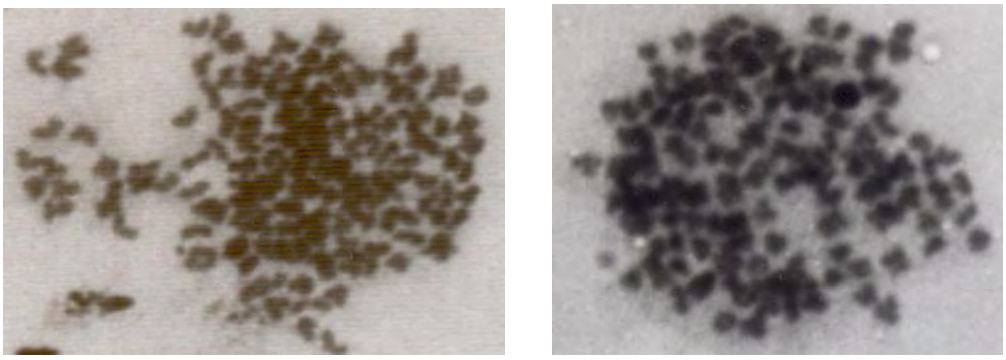
Table 5: Number of fishes for chromosome analysis

Fish species	Number of fish used for chromosome analysis	Number of slides used for chromosome analysis	Number of metaphase cells that were analyzed
Cyprinidae			
Labeobarbus intermedius	10	44	12
Labeobarbus nedgia	5	33	7
Labeobarbus brevicephalus	9	42	11
Barbus humilis	10	38	10
B. pleurogramma	10	38	8
Garra dembecha	8	28	8
Cichlidae			
Oreochromis niloticus	11	42	9

5 Results and Discussions

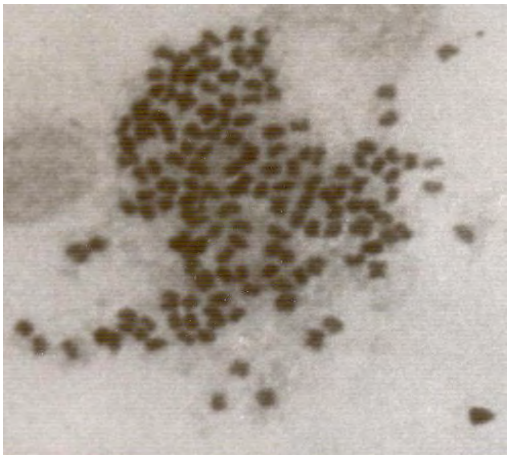
Chromosome number of the species *Labeobarbus intermedius, Labeobarbus nedgia, Labeobarbus brevicephalus, B. humilis*, *B. pleurogramma*, *Garra dembecha*, and *Oreochromis niloticus* are presented in Table 6 and (Fig. 5, 6, and 7). The chromosome counts were based on maximum counts from more than seven cells. Table 6 shows the summary of chromosome counts obtained from the species studied. Chromosome counts are the confirmation of chromosome count that were done by (Golubstov and Krysanov, 1993: Oellermann and skelton, 1990: and Berrebi and Rab, 1998) for *Labeobarbus intermedius* and reporting of chromosome number for *labeobarbus nedgia and Labeobarbus brevicephalus,* for small *Barbus* in general, by (Berrebi and Rab,1998; Dimmick *et al.,* 2001) and for *Oreochromis niloticus* by (Majumdar and Mc Andrew, 1986). Even though the confirmation is sure, because of the chromosomes are many in number, small in size, and lack of significant morphological differentiation between the chromosomes, it has not been possible to give detailed cytological characterization of most of the species including karyotype and number of satellite chromosomes. Thus, this made further cytological analysis difficult. It has to be noted that some chromosomal feature better observed under microscope than using photomicrograph.

5.1 Genus *Labeobarbus*

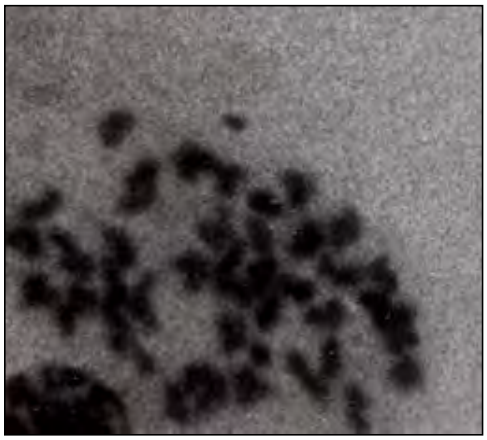


(A) *Labeobarbus intermedius* (B) *Labeobarbus nedgia*

Fig 5: Chromosome photograph of fishspecies of: (A) *Labeobarbus intermedius* (2n=150), (B) *Labeobarbus nedgia* (2n=150). Their magnification X1000

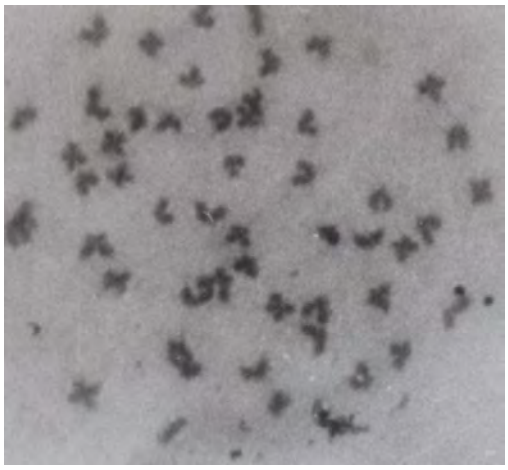


(A) *Labeobarbus brevicephalus*



(B) *B.humilis*

(C) *B. pleurogramma*



(D) *Garra dembecha*

Fig 6: Chromosome Photograph of fish species of:

(A) *Labeobabus brevicephalus* (2n=150), (B) *B. humilis* (2n=50), (C) *B. pleurogramma* (2n=50), (D) *Garra dembecha* (2n=50). Their magnification X1000.

5.1.3 *Labeobarbus intermedius*

Chromosome number 2n=150 was constantly found (Fig. 5-A). The chromosomes are smaller in size with homogenous appearance. The chromosome length within the complement appears fairly uniform. The reported basic chromosome number is x=25, from the genus. Previously reported chromosome numbers for several species of large *Barbus* is, 2n=150, for *Labeobarbus intermidus* (Golubstov and Krysanov, 1993; and Berrebi and Rab, 1998). Thus, the chromosome number 2n=150 found here for *Labeobarbus intermedius* probably indicates hexaploidy with basic chromosome number x=25. Therefore, this study helps for the conformation of the previous reports

Table 6 Summary of chromosome number counts for the studied species of the families Cyprinidae and Cichlidae.

Species studied	Number of fish analyzed	Code for the slides	Number of slide that metaphase cell were analyzed	Chromo some number
Cyprinidae				
Labeobarbus intermedius	13	LB.i	12	150
Labeobarbus nedgia	5	LB.ned	7	150
Labeobarbus brevicephalus	11	LB.brev	11	150
Barbus humilis	12	SB.hum	10	50
B. pleurogramma	12	SB.pleu	8	50
Garra dembecha	8	Ga.dem	8	50
Cichlidae				
Oreochromis niloticus	11	Oreo.n	9	44

5.1.2 *Labeobarbus nedgia*

Somatic chromosome number 2n=150 was found in many counts. (Fig.5-B), as chromosomes are large number and smaller sized were observed. Berrebi and Rab (1998) and Golubsov *et al.* (2001) reported the chromosome number for genus *Labeobarbus* is 2n=150. Thus, the chromosome number 2n=150 found here for *Labeobarbus nedgia* is a new finding which is simila to their reports.Therefore, this adds one chromosome number for this genus.

5.1.3 *Labeobarbus brevicephalus*

Somatic chromosome number 2n=150 was obtained from many counts (Fig.6-A). There is homogenous appearance of chromosome within a complement with fairly uniform length. Previously chromosome number reports of species in the genus of large *Barbus,* 2n=150 indicates polyploidy with possible basic chromosome number of x=25 (Golubstov and Krysanov, 1993: Berrebi and Rab, 1998; and Dimmick *et al.,* 2001). Thus, the chromosome number 2n=150 found here for *Labeobarbus brevicephalus.* Therefore, this study reports the chromosome number of this species the same chromosome number to the genus.

5.1.4 *B. humilis*

Chromosome number 2n=50 was found (Fig.6-B). It has relatively small chromosome number and size variation within a complement is evident. (Golubstov and Krysanov, 1993; Berrebi and Rab, 1998; and Dimmick *et al.,* 2001). Generally reported chromosome number of small barbs are diploid (2n=50). Thus, the chromosome number found here for *Barbus humilis* shows diploid with haploid chromosome number n=25. Therefore, this study confirms the previous reports.

5.1.5 *B. pleurogramma*

Chromosome number 2n= 50 was found (Fig.6-C). Chromosomes are relatively small in number and some morphological features are visible. Most of the chromosome appeared to have median centromeric position. According to Berrebi and Rab (1998), Golubstov and Krysanov (1993) from Lake Tana, and Dimmick *et al.* (2001) from Genale River, reported that smaller barbs are diploid (2n=50). Thus, the chromosome number found here for *Barbus pleurogrammas* probably indicates diploid, with haploid chromosome number n=25. Therefore, the present study confirms the previous reports.

5.2 **Genus *Garra***

5.2.1 ***Garra dembecha***

Chromosome number 2n=50 was found (fig.6-D). From observation of photomicrograph, it can be seen that the chromosome are relatively have visible morphological features. (Fig.6-D). Most chromosomes in a complement are metacentric or sub metacentric and some are acrocentric. Golubstov and Krysanov (1993) reported the chromosome number of he three *Garra* species (*G. dembeensis, G. makiensis* and *G. quadrimacukata*) from the freshwater of Africa were 2n=50. Thus, somatic chromosome number found here for *G. dembecha* are diploid, with haploid number of n=25. Therefore, this study presents the same chromosome number to other species in the genus *Garra.*

5.3 Genus *Oreochromis*

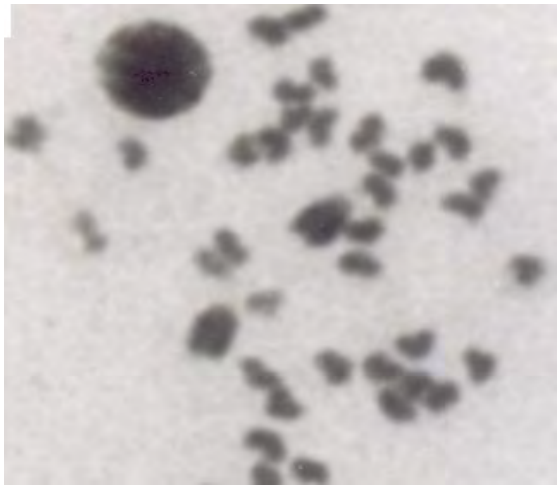


Fig. 7 Photomicrogrph of mitotic chromosomes (*Oreochromis niloticus*, 2n=44).

5.3.1 *Oreochromis niloticus*

Somatic chromosome number of 2n=44 was consistently found in many counts for this species (Fig.7).The chromosome are too small that morphological features are not evident.

Kocher *et al.* (1998) reported the karyotype of various Tilapia species which are highly similar, consisting of 22 pairs. In fact, only the two chromosomes are recognized, the remaining 20 being small in size and morphology. Thus, somatic chromosome number found here for O*reochromis niloicus* are diploid, with haploid number of n=24 and the chromosomes are small in size and similar in morphology except two large sized pairs. Therefore, this study shows the same result to that of the previous reports.

6. Conclusions and Recommendations

6.1. Conclusions

The present study, attempted to determine and and confirm the chromosome numbers and ploidy levels of some species of Cyprinidae and Cichlidae fishes. The small chromosome size and undifferentiation between chromosomes within the same complement made detailed cytological analysis difficult. Thus, this work could not go far beyond determining chromosome numbers.

Polyploidy is evident in some of the species. The observed chromosome numbers reveal that there are variable basic chromosome numbers for the two genera. Uniform chromosome size within a complement is observed in most of the species. This apparent uniformity in chromosome size could partly be due to the difficulty to study detailed structure of chromosomes as they are small in size.

As far as our literature survey goes the cytogenetic studies of fishes species of the family, Cyprinidae and Cichlidae, in Ethiopia is complet. Even if there are some chromosome number reports, their karyotype is not studied. Therefore, this work may add some valuable informations to our knowledge about the chromosome number of fish species found in Ethiopia in general and that of Lake Tana in particular. This work is far from complete for providing any clues about the evolution and phylogenetic relationships of the species in the two families, but it can serve as a starting point for further chromosome study of the families

6.2. Recommendations

More investigation need to be done on chromosome cytology of Cyprinidae from Lake Tana so as to make plausible inferences regarding the phylogenetic and evolutionary relationships between the small *Barbus* and large *Barbus* (*labeobarbus*) species and as well as within small *Barbus* and large *Barbus* species. Even if their smaller chromosome size and their larger number may be a hindrance it can be recommended that:

- Further chromosomal number study of many other species should be done for better understanding of cytogenetics of the families.
- Cytological techniques combined with molecular techniques are recommended to produce more information about the genetic diversity and phylogenetic relationships within the families of fishes in Lake Tana.

7. **Reference**

Abebe Getahun (2000). Systematic studies of African species of the Genus *Garra* (Pisces*:* Cyprinidae). Ph.D. Thesis, City University of New York, New York.

Abebe Getahun and Stiassny, M. (1998). Freshwater biodiversity crisis. *SINET: Ethio. J. Sci.:* **21**: 207-230.

Abebe Getahun, (2001). Lake Afdera: a threatened lake in Ethiopia. *SINET: Ethio.J. Sci*. **24** (1): 127- 131

Abebe Getahun, (2005b). An overview of the diversity and conservation status of the Ethiopia freshwater fish fauna. In proceeding of the pan-African Fish and Fisheries society, Contonoue, Benin, NOV. 2003.

Albertson, R.C., and Kocher,T.D. (2006). Genetic and developmental basis of cichlid trophic diversity. *Hereditas* **97**:211-221.

Berrebi, P. and Rab, P. (1998). *Barbus intermedius* species flock in Lake Tana (Ethiopia). III Cytogenetics and Molecular genetics data. *Ital. zool.* **65**:15-20.

Berrebi, P. (1995). Speciation of the genus *Barbus* in the south Mediterranean basin. Recent advances from biochemical genetics. *Biol. Cons*. **72**:237- 249

Blaxhall (1975). Fish chromosome techniques of a review selected literature. *Fish Biol.* **1**: 315-320.

Bond, E.C. (1996). *Biology of Fishes*. Saunders College publishing, U.S.A.

Boulenger, G. A. (1902). Description of new fishes from the collection in Abyssinica. *The Annal and Magazine of Natural History, 7th series.* **10**(62): 421-437.

Boulenger, G. A. (1911). Catalogue of the freshwater fishes of Africa. British Museum.**2**:1-519.

Chorowicz, J., Collet, B., Bonavia, FF., Mohr, P.,Parrot, JF.,and Kome, J. (1998). The Tana basin, intraplateau uplift, rifiting and subsidence. *Tectonophysics.* **295**:351-367.

Cohn, N. S. (1969). *Element of Cytology.* Harwurt, Brace and World, Inc., New York.

Daget, J. Gosse, G., Teugels and Thys Van den Audenaede(1991). *Checklist of freshwater fishes of Africa*, CLOFFA,4, Bruxelles ISNB, Tervuren: MRAC, Paris: ORSTOM.

De Graaf, M. (2000). The piscivoous *Barbus* of Lake Tana (Cyprinidae, Ethiopia). Major question on their evolution and explotation. *Netherland J Zool*. **50**(2): 215-223.

De Graaf, M. (2003). Lake Tana's piscivoous *Barbus* (Cyprinidae, Ethiopia). ecology, evolution, explotation. Doctoral Thesis. Exprimental group, Wageningen University, Wageningen. The Netherlands.

Demeke Admassu and Elias Dadebo (1997). Diet composition, length weight relationship and condition factor of *Barbus* species Ruppell 1836 (Pisces: Cyprinidae) in Lake Awassa, Ethiopia. *SINET*: *Ethiop. J. Sci.* **20**(1): 13-30.

Demirok, N. K., and Onlo, E. (2001). Karyoypes of cyprinid fish *Capoeta truta and Capoeta capoeta umbla* (Cyprinidae) from he Tigris river. *Turk. J. zool.* **25**: 389-393

Dimmick, W. Benedzen, P. and Golubstov, A. (2001). Genetic compositionof the three *Barbus* (Cyprinidae) morhpotypes from the Genale River, Ethiopia. *Copeia.* **4**:1123-1129.

Doadrio, I**.** (1994). Freshwater fish fauna of North Africa and its biogeography. *Ann Mus R Afr Centr Zool,* **275**: 21-34.

Eshete Dejen (2003). *Ecology and potential for fishery of the small barbs* (Cyprinidae Teeostei) *of Lake Tana, Ethiopia*. Pp. 180. Ph. D Thesis, Agricultural University. Wageningen. The Netherlands.

Eshete Dejen, de Graaf, M. and Sibbing, F.A. (2000). *Barbus tanapelagius* a new species from Lake Tana (Ethiopia); its morphology and ecology. *Env. Biol. Fish.* **59**; 1-9.

Eshete Dejen, de Graaf, M. and Sibbing, F.A., Nagelkerke, L. A.,Osse,T. W.and Rutjes, H.A. (2002). The small *Barbus humilis* and *B. trispilopleura* of Lake Tana (Ethiopia); are they ectotypes of the same species? *Env. Biol. Fish*. **65**:373-386.

Garber, E. (1992). *Cytogenetics, An Introduction.* pp-259. Tata Mc Gram-Hill. New Delhi

Genanaw Tesfaye (2006). *Diversity, relative abundance, and biology of fishes in Angereb and Sanja Rivers,Tekeze basin, Ethiopia.* Msc. Thesis, Addis Ababa University. Addis Ababa, Ethiopia.

Getachew Tefera (1987). A study on an herbivorous fish, *Oreochromis niloticus* diet and quality in two Ethiopian rift valley lakes, Awassa, Zwai. *J. of Fish Biol.* **30**:239- 244.

Gold, J.R. (1979). A fast and easy method for chromosome karyotyping in adult teleosts *Fish Biol. Research Faculty.* **36**:169-171.

Gold, J.R. (1979). *Fish physiology.* Volume III by Academic Press. pp. 353-386.

Golubstov, A.S. and Krysanov, E.Y. (1993). Karyological study of some Cyprinid species from Ethiopia. The polyploidy differences between large and small Barbus of Africa. *Journal of Fish Biol*. **42**:445-455.

Golubstov, A.S., and Mina, M.V. (2003), Fish species diversity in main drainage system of Ethiopia *Ethio. Na. Sci.* **5(2):**281-31.

Golubstov, A.S.and Krysanov E.Y. (1996). Karytypes of some Ethiopian *Barbus* and *Varicorhinus* from the Nile Basin including Lake Tana morphotypes. *Folia Zoologica.* **45**(Supp. 1): 67-75.

Golubtsov, A.S., Dgebuadze, Yu.Yu. and Mina, M.V. (2002). *Fishes of Ethiopian Rift Valley*. Pp. 167-258. **In**: C. Tudorancea and W.D.Taylor (eds.) *Ethiopian Valley Lakes*. Backhuys Publishers, Leiden, Holland.

Graham, GW., Black, RP. (1925). Report to the mission to Lake Tana. 1920-1921. Government press. Cairo.

Guegan, J.F., Rab, P.,Machordom, A., and Doadrio, I. (1995). New evidence of Hexaploidy in ''Large'*Barbus* (Cyprinidae,Teleostei) with some considerations on the origin of Hexaploidy. J. *Fish. Biol*. **47**:192-198.

Guegan, J.F., Rab, P.,Machordom, A., and Penndicus, A. (1995). Karyotype of the three Small *Barbus* (Cyprinidae) from Republic of Guinea (West Africa) with a review on karyology of African Small *barbus. Karyologia.* **48**(3-4): 299- 307.

Helfmann, G.S., Collete, B.B, and Facey, D.E (1997). *The diversity of fishes*. Pp., 273-294. *Blackwell Science*.., Blackwell, Malden, U.S.A.

Howes, G. J. (1991). *Systematics and Biogeography*: An Overview in Cyprinid Fishes, Systematics Biology and explotation . Chapman and Hall. London.

Johnson TC.,Scholaz,CA.,Talbot MR. (1996) Late Pleistocene desiccation of Lake Vicoriaand rapid evolution of cichlid fishes. *Science* **273**:1092-1093.

Karl, F.L., John,.E.B., Robert, R.M.,and Dora R. May Passino (1977). *Ichthyology.* John Wiley and *Sons*. New York, London.

Kocher TD (2004). Adaptive evolution and explosive speciation: the cichlid fish model. *Nat. Rev Genet* **5:**288-298.

Kocher, T., Lee, W., Sobolewska, H. Penman, O. and Mc Andrew, B. (1998). A Genetic Linkage Map of Cichlid Fishes, the tilapia *(Oreochromis*

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

Leveque, C. (1997). *Freshwater Biodiversity Dynamics and Conservation: the fish of tropical Africa.* Pp., 331-341. The Rift Building Trumpingston, Cambridge.United Kingdom.

Lowe-McConnell, R.H. (1991). Ecology of Cichlidae in South America and Africa waters excludingCreater Lake., **In**: *fish and fishery series 2*, pp.60-85. Chapman and Hall. London. *Conservation Biology*. **71:**634-643.

Mohr, PA. (1965). The geology of Ethiopia, University College, Addis Ababa, press. Addis Ababa, Ethiopia.

Moyle, P.B., and J.J. Cech. Jr. (1988). *Fishes- An introduction to Ichthyology*

Nagelkerke, L.A, Sibbing, FA., Boogaart, JGM., Lammens, EHHr, Osse, JWM, (1994). The barbs (*Barbus spp)* of Lake Tana: a forgotten species flock? *Env. Biol. Fish*.**39**: 1-22.

Nagelkerke, L.A. (1997). *The barbs of Lake Tana, Ethiopia: Morphological diversity and its implication for taxonomy, trophic resources partitioning and fishries*. Ph. D Thesis, Agricultu University Wageningen. The Netherland.

Nagelkerke, L.A., and Sibbings, F. A. (1998). The *Barbus intermedius* flock of Lake Tana, (Ethiopia). The ecological and evolutionary significance of morphological diversity. *Ital. J. Zool.* **65**(supp. 1): 3-7.

Nagelkerke, L.A., and Sibbings, F. A. (2000). The large barb (*Barbus spp.,* Cyprinidae, Teleostei) of Lake Tana (Ethiopia), with the description of new species, *Barbus ossenisis. Neth. J. Zool.* **50**:179-214.

Nagl, S., Tichy, H., Mayer, W., Takezaki, N., Takahata, N., Klein, J. (2000). The origin and age of haplochromine fishes in lake Victoria, East Africa. *Proc.R.Soc.Lond.B*. **267**:1049-1061.

Nelson, J. S. (1994). *Fishes of the world*, 3rd edn. John Wiley and sons, New York.

niloticus). *Genetics***.148**:1225-1232.

Nogusa, S. (1960). A comparative study of the chromosomes in fishes with

Olleerman and Skelton (1990). Hexaploidy in yellow fish species (*Barbus,* pisces, Cyprinidae) from South Africa, *Can.J. Zool* .**68:**1124-1131**.**

Oziut-Costaz and Foresti (1992). Fish Cytogenetic Research:Advance Application and Perspectives. *Neth. J. Zool.* **42**:277-290.

particular considerations on taxonomy and evolution. Mem.Hyogo Univ. Agric. (Hyogo Noka Daigaku Kiyo). *Biol. Ser.* **3** (1):1-62

Post, A. (1973). Chromosome of two fish species of the genus Diretimus, (Osteichthyes, Beryciformes: Diretimidae). **In:** *Genetics and Mutagenesis of fish.* pp. 103-111. Springer verlang. Berlin and New York..

Roberts, F.L. (1967). Chromosome cytology of the Osteichthyes. *Prog. Fish Cult.* **29:**75-83.

Roberts,F.L. (1975). Geographocal distribution of Africa freshwater fishes. *Zool. J. Linn.Sc.* **57**:249- 319

Robertson,W.R.B.(1916). Chromosome studies, Taxonomic relationships show in chromosomes of Tettgidae and Acrididae.V-shaped chromosomes and the significance in Acrididae, Locustidae , and Grgllidae. *J. Morphol*. **27:**179-332.

Shibru Tedla and Fisseha H/Meskal (1981). Introduction to transplantation of freshwater fish species in Ethiopia. *SINET: Ethiop.J.Sci***. 4:**69-72

Shibru Tedla. (1973). *Freshwater Fishes of Ethiopia*. Haile Selassie I University; pp. 101. Dept. Biology, Addis Ababa, Ethiopia.

Sibbing, FA. and Nagelkerke, LAJ. (2001). Resources Partitioning by Lake Tana barbs predicted from fish morphometric and prey characteristics. *Rev.Fish Biol. Fisher.***10**: 393-437.

Skeleton, P.H. (2001). *A Complete Guide to the Freshwater Fishes of Southern Africa* Struck, Cape Town.

Skelton, P.H. (1970). *A Complete Guide to the Freshwater Fishes of Southern* Africa. Pp.338. southern Book Publishers, Halfway House. London.

Skelton, P.H., Tweddle, D. and Jackson, P. B. (1991). Cyprinids of Africa, pp. 211-239, **In:** Winfieds,J. J. and Nelson J. S. (eds) *Cyprinid fishes, systematics, biology and exploitation*, Chapman and Hall. London.

Soltis DE, Soltis PS**.** (1999). Polyploidy: recurrent formation and genome e evolution. *Trends Ecol Evol,* **14:** 348-352.

Springer (2007). Global diversity of fish (Pisces) in fresh water. *Hydrobiologia*. **595**(1):545-567.

Tesfaye Wudneh. (1998). *Biology and management of fish stocks in Bahir Dar Gulf, Lak Tana, Ethiopia*. Ph. D. Thesis, Agricultural University Wageningen , The Netherland.

Teugels, G. G. (1986). A systematic revision of the African Species of the genus *Clarias* (pisces, *Claridae*). Annaels du Museed Royal dl Afrique Centerale. *Sciences Zoologiques*, *Ser*. In-80,**247:**1-1999.

Tsigenopoulos, CS., Rab, P., Naran, D., Berrebi, P. (2002). Multiple origin of polyploidy in the phylogeny of southern African *Barbus* (Cyprinidae) at inferred from mtDNA markers. *Heredity.* **88**:466-473.

Uyeno, T. and Miller, R. (1971). Multiple sex chromosomes in Mexican Cyprinodontid Fish. *Nature.* **231**:442-452.