INTRODUCTION AND HISTORICAL REVIEW

Production of animal protein foods in Egypt is comparatively low and is far less than the needs of its tremendous rate of increase in human population. This critical situation has stimulated interest in the search for new resources of such foods. The role of agriculture in the production of animal protein food may be extended and supplemented by aquaculture. Aquaculture is the controlled production of water organisms, mainly fish, crustaceans, molluscs and algae **(FAO, 1984; GAF, 1997; Haroun, 1999 and Salama, 2001)**.

The available living resources for aquaculture and fisheries are mostly to be found in wild populations. There is a wide array of aquatic species to be accurately identified and their populations to be characterized for evaluation of aquaculture potential. In Africa, the home of tilapias, and where they are under investigation for farming, the need to characterize and to name tilapias, consistently and accurately is extremely important for consumers, farmers and researchers **(Pullin, 1996 and Beveridge & McAndrew, 2000)**.

Family Cichlidae is naturally distributed throughout Africa, Central America up to Mexico, the northern half of South America and parts of India **(Fryer & Iles, 1972)**. Present world distribution of tilapias covers the areas between the 20^{o}C winter isotherms. It extends to southern USA, Europe and Far East (**Balarin & Hatton, 1997 and Haroun, 1999)**. In Egypt, the tilapia fishes are naturally distributed throughout the River Nile and its branches.

The African cichlid fish radiations are the most diverse extent of animal and provide a unique system to test predictions of speciation and adaptive radiation theory. The past few years have seen major advances in the phylogenetic, evolutionary, biogeography and ecology of cichlid fish **(Seehausen, 2006)**.

Tilapias represent the most important group of family Cichlidae and these fishes are important for the nutritional and socio–economic development of tropical and subtropical regions, especially in Egypt. Also, it is important for aquaculture research in general **(FAO, 1980; Pullin, 1985; Oberst *et al*., 1992 & 1993; Rajavarthini *et al*., 2000; Morals *et al*., 2001 and Sharaf Eldeen & Abdel Hamide, 2002)**.

Tilapia is the common name for over 70 species of perch-like fishes (Family: Cichlidae) native to the fresh water of tropical Africa **(Trewavas, 1982; Stiassny, 1991 and Stiassny *et al*., 1992)**. The term is derived from the African native Bechuana word "thiape" meaning fish. **Naylor *et al*. (2001)** were first bred thousands of years ago in ancient ponds and aquaria for the temples of Egyptian pharaohs.

In Egypt, there are three genera of tilapia, according to the breeding habits, including four species. The genera include the biparental and parental mouthbrooding genus *Sarotherodon* [*Sarotherodon galilaeus* **(Günther, 1862; Boulenger, 1899 and Trewavas, 1984)]**, maternal mouthbrooding genus *Oreochromis* [*Oreochromis niloticus* **(Linnaeus, 1758; GÜnther, 1864; Steindachner, 1864; and Trewavas, 1984)** and *Oreochromis aureus* **(Steindachner, 1864; Boulenger, 1899; Daget, 1954; Blache &**

Miton, 1960 and Trewavas, 1965)] and the substrate spawning genus *Tilapia* [*Tilapia zillii* **(Smith, 1840; Gervais, 1848; Günther, 1859, 1862 & 1864; Steindachner, 1870; Boulenger, 1899 and Trewavas, 1984)]**.

All tilapias exhibit a high degree of parental care and according to this function they are sharply divided into substrate spawners, or guarders of the brood on one hand and mouth brooder on the other **(Lagler *et al*., 1977)**. The substrate spwaners constitute the genus *Tilapia* whereas; the mouth brooders constitute the genera *Oreochromis* and *Sarotherodon*.

It is vital that all those who work for the conservation and sustainable use of living organisms are able to describe them accurately, in terms of their identity, diversity and abundance. Most biologists regard the first significant system, describing living organisms as species and giving them a standardized nomenclature, to be that devised by **Linnaeus (1758)**.

Living resources, whether for present of future use, are in effect genetic resources, within the species communities and ecosystems that house them. Many of their useful attributes are coded for by genes, therefore all concerned with their conservation and use need to be able to determine their identity: at least at the species level, through the branch of science known as Taxonomy or Systematics, both using standard methods and descriptors **(Gyllensten, 1985; Falk *et al*., 1996; Vari, 2003; Anderson *et al*., 2008 and Goldstein, 2009).**

The study of morphometric and meristic characteristics of species is a valuable tool for the differentiation between their

populations whereas study and analysis of size and shape is considered as a basic step in the study of biometric variations in populations of organisms **(Jolicoeur & Mosimann, 1960)**.

The bulk of systematic information in fish, including tilapias is based on measurements and counts of comparable body parts and characteristics **(Thys van den Audenaerde, 1970; Trewavas, 1984; Lévèque & Paugy, 1984 and Teugles & Thys van den Audenaerde, 1992; Henry *et al*., 2005; Leitner & Bulak, 2008 and Hoening *et al*., 2008)**. The theory practice and results of this is known as morphological systematics. The analysis of both morphometrics and meristic characteristics can separate species but not strains or hybrids **(Pante *et al*., 1988)**.

Although meristic variations are broad, their usage in species identifications is more preferable than that of morphometric indices; the validity of such usage should be more amplified if the genetic and nongenetic variations on intra- and interspecific levels are considered (**Mekkawy, 1987 and Mekkawy *et al*., 2002)**.

Morphological and meristic characteristics as well biochemical analysis were considered as classical methods for identifying fish species. Morphological and meristic characteristic analyses were used to identify two species of *Pagellus* (Family, Sparidae) **(Wadie *et al*., 1999)**. Also, morphologic analysis were made on chub mackerel fish (*Scomber* & *Oponicus*) collected from two areas of the south west Atlantic Ocean and one from the Mediterranean sea **(Roldan *et al*., 2000)**.

The concept of size and shape on the morphometric characteristics of the four *Siganus* species *(S. rivulatus, S. luridus, S.stellatus and S. argenteus)* was applied to elucidate intra – and inter-specific variations and to determine their validity in stock unit identification of these species **(Obady, 2003)**. As well as two to four population of hilsa, *Tenualosa ilisha* in Bangladesh has been identified using morphlogical measurements and meristic counts **(Salini *et al*., 2004)**.

The population differentiation of seven flat fish species *Citharus linguatula, Lepidorhombus boscii, Platichthys flesus, Microchirus azeria, Solea lascaris, Solea senegalensis* and *Sole*a *sole*a, widely distributed along the eastern north Atlantic were investigated using morphometric and meristic characterstics as a morphometric analysis **(Marques *et al*., 2006)**.

For *Tilapia* species, the morphological identification is greatly complicated by the extensive intraspecific variation of the morphological characteristics used for classical identification **(Albertson *et al*., 1999)**. Moreover, the specific identification of *Tilapia* species is required for detection and investigation among populations **(Trewavas, 1984 and Teugles & Thys van den Audenaerde, 1992)**. Also, morphometric and meristic characteristics were used to evaluate and describe three *Tilapia* species (*Sarotherodon, melanotheron, Oreochromis niloticus and Oreochromis aureus* **(Yapi- Gnaoré, 2001)**.

The widespread distribution of many species makes the same species confusing under quite different conditions to those in which

they are originally described **(McAndrew & Majumdar, 1983 & 1984)**. The major obstacle in tilapia culture identification is the species richness of all *Tilapia* genera, accompanied by striking similarities and variations of morphological and meristic characteristics among species. This overlapping of morphological and meristic characteristics and striking similarities among species resulted from the high levels of interference of fish genomes **(Trewavas, 1984; Teugles & Thys van den Audenaerde, 1992 and Kocher *et al*., 1998)**.

Morphological description and morphometric analysis were used to elucidate inter specific variations and to determine their validity in identification of *Tilapia* species **(Anene, 1999 and Azab, 2003)**.

As the scales are most external to the body of fish, they continuously come in contact with water and the pollutants therein. Therefore, these can be very good bio-indicators of the state of pollution in water bodies. Also, scales have been used for classification and identification of growth in studies of different fishes. But the fine structure of fish scales, including their structural demarcations and shape, has been used inconsistently in fish taxonomy **(Al-Zahaby *et al*., 1990; Lippitsch, 1990 and Rishi & Jain, 1998).**

Detailed structure of the fish scale can be helpful in the identification of fishes up to major groups and species levels, phylogeny, sexual dimorphism, age determination, past environment experienced by fish, discriminating between hatchery reared and wild

populations, migration, pathology of fish scale due to water pollution of the water body and for the growth studies **(El-Serafy *et al*., 1992; Lippitsch, 1992 & 1993; Johal and Agarwal, 1997; Jawad, 2005 and Esmaeili & Gholami, 2007).**

The dermal derivatives of fish body are important structures used as versatile research material in fisheries. They exhibit distinct pattern of dark and light bands corresponding to closely and widely spaced circuli that form annular zones depicting the age of fish in years. The engraved pattern of circuli on scale serves as a blueprint for the physiological epochs of fish life, narrating its growth history. Besides this established role of scales in fish biology, these have numerous hidden details in their sculptural design that contribute effectively to fish identification and classification. So, an attempt has been made to define structures that can contribute towards species discrimination among four species of *Labeo* (*L. calbasu, L. rohita, L. gonius* and *L. bata*) using Scanning Electron Microscopy (SEM) **(Kaur & Dua, 2004).**

SEM technique was used to describe the morphology of the otoliths in the blue marlin **(Radtke *et al*., 1982).** SEM investigation of posterior-lateral parts of teleostean fish scales showed that the membranes of their surface epithelium are covered by complicated system of tuberculi and ridges, forming a maze-system over their cells. These ridges form patterns on the cell membrane that seem to be species-specific. The idea behind this comparative investigation is the possibility of using sub micro-structures for taxonomic- evolutionary as well as ecological studies **(Fishelson, 1984).**

SEM had facilitated the application of microstructures to systematics. Although both systematical and functional approaches utilize the surface structure of the scales, the ontogenetic development of these superficial ornamentations has been studied in detail scale structure as well as the function of associated cells in scale production of the cichlid *Hemichromis bimaculatus* **(Sire, 1986).** Furthermore, the region between the epidermis and the surface of the overlapping part of scales has been studied in two cichlid teleosts using transmission electron microscopy **(Sire, 1988).** Based on light microscopic examination of scale structures, the surface structure of the scales of *Oreochromis mossambicus* and *Oreochromis niloticus* was studied by using SEM **(Gabr and Sakr, 1992).**

The recent revival of interest in the superficial ornamentation of teleost scales involves also the functional approach in addition to the systematic one. **Mekkawy *et al.* (1999)** studied the scale characteristics of five species of genus *Epinephelus* in term of morphometry and SEM techniques. By using the same techniques, **Harabawy (2002)** studied the surface structure of the normal & lateral line scales of seven *Lethrinus* species, to estimate the species-specific variations in shape of inter-radial tongues and the first circuli delimiting them, form of inter-radial denticles, the rostrolateral circuli with their grooves and denticles and the lateral line canal along the lateral line.

The microstructure of fish scales extracted from sea bream, *Pagrus major*, was reported using SEM, transmission electron microscopy (TEM), energy-dispersive X-ray analysis (EDX), X-ray

diffraction (XRD) and Fourier transform infrared (FTIR) spectroscopy **(Ikoma *et al.*, 2003).**

An attempt has been made to study the ultra structure of the scale of cobitid, *Cobitis linea* employing different modes of SEM, such as secondary electron image (SEI, low energy electrons), back scattered electron image (BEI, high energy electrons), mixed signals of both SEI and BEI and reverse polarity have been employed for the study of scale morphology and for the details of the circuli **(Reddy, 2006)**.

Shape, size and number of scales are suitable tools in fish taxonomy. The importance of scale morphology used in classification was strengthened with the introduction and development of SEM. So that, scales of many different fish species have been studied using SEM **(Jawad & Al-Jufaili, 2007).** The normal and lateral line cycloid scales of cyprinid fish *Capoeta damascina* have been subjected to the SEM technique in order to study their detailed structure **(Esmaeili *et al.*, 2007).**

Concerning the importance of scale morphology in fish taxonomy, scale ultra-structure of tank goby fish *Glossogobius giuris* was studied using SEM. Variations are found in scale morphology of this fish, in which, the position and shape of focus, absence of lepidonts and arrangement of cteni on the scales could be used as important taxonomic characteristics **(Esmaeili *et al.*, 2009).**

Nano-scale structural analyses of biomineralized materials can frequently help elucidate important structure-function relationships in these complex organic-inorganic composites. Atomic Force

Microscopy (AFM) imaging of the exterior and interior of fresh trabecular bone taken from a bovine vertebrae reveal a woven layer of lightly mineralized fibrils on the surface of the trabecula and an interlocking network of more heavily mineralized fibrils in the trabecular interior **(Hassenkam *et al.*, 2004).**

There is current interest in studies of objects with nanometer-scale ordering, particularly, photonic crystals, i.e., structures with periods comparable to the wavelength of the incident electromagnetic radiation. The AFM, spectroscopy and X-ray analysis were applied to specimens of molluscan shells, peacock feather barbules and human horny tissue to characterize each species **(Il'inskii *et al.*, 2006).**

For higher resolutions, the scientists resort to AFM, which can be conducted in nearly physiological condition. This technique not only yields qualitative information on the surface topography and mechanical properties but also qualitative data on the surface component dimensions. It will enable accurate imaging of nano-asperities and nano-grains or distinguish between softer/sticker zones (covered with the organic matrix) and harder (mineral) ones. AFM imaging in ambient conditions was employed to produce surface topographical images of freshly cleaved nacre samples of gastropod mollusks shell of *Trochus niloticus* and *Haliotis rufescence* **(Bruet *et al*., 2008).**

Microcomputed topography was used to obtain AFM full three-dimensional constructs of both a single scale and multiple interlocking scales of armored fish and provided information on the density

distribution of the flexible joints between two scales and the scale-to-scale and scale-to-body interfaces **(Song *et al.*, 2007).**

The general interspecific osteometric differences between four *Siganus* species were reflected by the X-ray photographs. The photographs reflect the differences in skull, body cavity shape, vertebral column, and pelvic and pectoral girdle characteristics as well as the interrelationship between the positions of different fins relative to the axial skeleton **(Obady, 2003).**

The persistence of tilapia (*Oreochromis* spp.) in polluted waters and the development of a suite of morphological deformities can be investigated by X-ray photographs. These photographs were used to study the endoskeleton morphological deformities that could be used as biomarkers of contamination **(Sun. *et al*., 2009).**

The pharyngeal and oral teeth of the fish *Tilapia mossambica* were examined with a scanning microscope. It appeared that the dorsal pharyngeal teeth form a peculiar hooklike extension at the tip, whereas, the ventral pharyngeal teeth tend to curve in a posterior direction. The two lateral flanges at the tip of the ventral teeth are probably the areas of contact with the dorsal teeth when the latter are pressed down during sound production or feeding. However, the oral teeth develop along a different line. Apart from villiform teeth the upper and lower jaws also develop tricuspid and bicuspid oral teeth, with the bicuspids concentrated mainly along the outer edge of the jaw **(Lanzing & Higginbotham, 1976).**

The occurrence of axons with certain neurochemical markers in gingival and teeth in the lower jaw of the cichlid *Tilapia mariae* was

examined by immunohistochemistry. It was found that gingival and pulpal axons can be labelled with antibodies against calcitonin gene – related peptide, substance P, tyrosine hydroxylase, neuropeptide Y, choline acetyl transferase and vasoactive intestinal polypeptide **(Tuisku & Hildebrand, 1996).** Both gingival and dental domains in *Tilapia mariae* contain axons with sensory and autonomic chemical phenotypes, the occurrence and distribution of which are generally similar to the mammalian counterpart.

Four *Siganus* species were identified according to the teeth features in lower jaw and the upper pharyngeal teeth features in upper jaw **(Obady, 2003)**. Because cichlid adult teeth are replaced every 100 days **(Tuisku & Hildebrand, 1994 and Huysseune & Sire, 1998)**, odontogenesis can be studied at any life stage.

The dramatic differences in oral jaw teeth morphology were used to identify closely related species of cichlid: an adaptive trait **(Albertson & Kocher, 2001),** (*Metriaclima zebra* and *Labeotropheus fuelleborni*) and their F1 hybrid **(Albertson *et al.*, 2003a)**. Cichild species, also, have distinctive teeth shapes, a phenotypic character which has been difficult to study in other model systems **(Streelman *et al.*, 2003a).** The differences in phenotypic character were controlled by a single major gene **(Albertson *et al.*, 2003b and Kocher, 2004).**

The genus *Oreochromis* has been divided by **Trewavas (1984)** into five subgenera: *Orechromis, Alcolapia, vallicolla, Nyasalapia* and *Neotilapia*. The distinguishing characters of the subgenera include the size, shape and number of tooth cuspids; shape of the preorbital

bone; number of openings in the preorbital bone; number of anal spines; relative size of belly scales; size and shape of pharyngeal teeth.

Advances in molecular biology techniques have enabled the direct analysis of the nucleotide sequences of the DNA contained either inside the nucleus (nDNA) or in the mitochondria (mtDNA). Advantages of DNA analysis compared to protein data include the possibility of examining DNA regions with different degrees of mutation rate, as well as, detecting mutations that do not change the amino acid sequence and facilitating the detection of genetic divergence among populations **(Kaneko *et al*., 2002 and Ledee *et al*., 2003; Bosworth & Farrell, 2006; Tiano *et al*., 2007 and Chen *et al*., 2009)**.

The Polymerase Chain Reaction (PCR), which was developed at the cetus corporation in Emeryville, California, USA, and employs the enzymatic amplification of DNA in vitro **(Saiki *et al*., 1985 and Mullis & Faloona, 1987)**, also has been useful in resolving the complex taxonomy and identification process of the genus *Acanthamoeba* **(Fry *et al*., 1991; Riley *et al*., 1991 and Vodkin *et al*., 1992)**.

Nucleolin, the most abundant non-ribosomal protein, has been associated with several steps in ribosomal biogenesis process. These include chromatin structure remodeling, regulation of rDNA transcription, rRNA maturation, ribosomal assembly and nucleus cytoplasm transport **(Bouvet *et al*., 1998; Ginisty *et al*., 1998 & 1999 and Roger *et al*., 2002 & 2003)**. Two carp nucleolin genes were

identified and the phylogenetic analyses were compared between different species **(Quezada *et al*., 2006)**.

Ribosomes are composed of 3-4 rRNA molecules and about 60-80 protein subunits. Ribosomal protein (r- protein) genes have been considered housekeeping genes, with a strongly coordinated constitutive expression that is closely linked to that of the different ribosomal RNA to form the entire ribosome. Ribosomal protein expression in adjusted according to the needs of the cell for protein biosynthesis **(Kraakman *et al*., 1991)**.

The coordinated expression of the r- protein genes is controlled at various levels. In prokaryotes, most of them are organized in a comparatively small number of operons, which encode a repressor of both transcription and translation that binds to a specific site on the polycistronic RNA **(Nomura, 1999)**. In eukaryotes, r- protein gene specific regulatory elements have been identified in the different promoter sequences, consistent with a coordinated expression. Most eukaryotic r- protein genes have special short promoters devoid of a TATA box. The transcription start site is part of an oligopyrimidine tract flanked by GC- rich sequences **(Hariharan & Perry, 1990 and Vera *et al*., 2003)**.

Studies of ribosomal RNA genes have gained prominence in abroad range of animals and plants, especially for the identification of species and specific populations, the study of evolutionary relationships and the characterization of genome structure. Variations in the sequences of 5S rRNA, 18S rRNA and 28S rRNA genes owing to insertions, deletions and mini-repeats have been useful for

evolutionary studies and served as species or population specific markers for various organisms including plants **(Zanke *et al.*, 1995)**, mammals **(Suzuki *et al.*, 1996)** and fishes **(Pendás *et al.*, 1995; Nieddu *et al.*, 1998; Céspèdes *et al.*, 1999; Carrera *et al.* 2000; Martins *et al.*, 2002 and Kumar *et al.*, 2009)**.

The reverse transcription of small subunit ribosomal RNA was used to determine the partial nucleotide sequences of small subunit ribosomal RNA to characterize seven isolates of *Acanthamoeba* representing all three morphological groups **(Johnson *et al.*, 1990)**. The phylogenetic relationships of *Acanthamoeba* have been studied using PCR to compare the small subunit ribosomal RNA gene sequences **(Sogin, 1989 & 1991; Wainwright *et al.*, 1993; Awwad, 1995 and Ledee *et al*,. 2003)**. While as a taxonomic relationship of *S. difficile* and *S. agalactiae* was supported by using the comparative nucleic acid sequence analysis of 16S – 23S ribosomal DNA (rDNA) intergenic spacers **(Berridge *et al.*, 2000)**.

Sequencing variation in the 18S rRNA gene of *Plasmodium ovale* was detected **(Kawamoto *et al.*, 1996)**. Analysis of the target sequence in the 18S rRNA gene indicated that in the DNA of two isolates from patients three nucleotides in the probe region from the typical *P. oval* sequence were different, with deletions of two nucleotides and the substitution of one nucleotide **(Calderaro *et al.*, 2007)**.

DNA from four monogenean species of the genus *Lamellodiscus* and their three fish host species from the genus *Pagellus* were sequenced in order to estimate the molecular divergence and the

coevolutionary interactions in this association (**Desdevises *et al.*, 2000)**. The phylogenetic relationships among *Lamellodiscus* species were estimated with partial 18S ribosomal DNA sequences while mitochondrial cytochrome *b* DNA sequences were used for their fish hosts.

Thirty–one gyrodactylid species (Gyrodactylidae: monogenea) from five families of freshwater fish (teleostei) were examined and variable region V_4 of the 18S small subunit ribosomal RNA gene and ribosomal RNA Internal Transcribed Spacers (ITS1 and ITS2) were sequenced. Both the V_4 region and spacers (ITS1 and ITS2) proved useful for gyrodactylid diagnosis. Sequences of these fragments exhibited interspecific variations and allowed clear determination at the species level **(Matejusova *et al.*, 2001).**

Molecular evolution of the nucleotide sequences of 18S ribosomal RNA genes from set of nematodes in the family Rhabditida was investigated **(Fitch *et al.*, 1995)**. Also, Restriction Fragment Length Polymorphism (RFLP) analysis of 18S rRNA gene was identified human pathogen *Shistosoma* sp. **(Awwad & Morsy, 2001).** The data revealed that, 18S rRNA RFLP and sequences are likely to be a useful tool to resolve relationships at intra-familiar level.

Members of phylum Acanthocephala are parasites of vertebrates and arthropods, and are distributed worldwide. The phylum has traditionally been divided into three classes, Archiacanthocephala, Palaeacanthocephala, and Eoacanthocephala; a fourth class, Polyacanthocephala, has been recently proposed. However, erection of this new class, based on morphological characters, has been

controversial. **Garcláa-varela *et al*. (2002)** sequenced nearly complete 18S rRNA gene of Polyacanthorhynchus caballeroi (Polyacanthocephala) and Rhadinorhynchus species. These sequences were aligned with another 21 sequences of acanthocephalans representing the three widely recognized classes of the phylum and with 16 sequences from outgroup taxa, Phylogenetic relationships inferred by maximum-likelihood and maximum-parsimony analyses showed Archiacanthocephala as the most basal group within the phylum. Whereas, classes Polyacanthocephala and Eoacanthocephala formed a monophyletic clade, with Rhadinorhynchus as its sister group, these results are consistent with the view of Polyacanthocephala representing an independent class within Acanthocephala.

Nearly complete 28S and 18S rRNA genes sequences (>3852nt) used to classify and evaluate the phylogenetic relationships of Chilopoda (Myriapoda, Arthropoda) **(Giribet *et al*., 1999)** and Ecdysozoa (molting animals), especially Phylum Arthropod and Phylum Kinorhyncha (mud dragons) **(Mallatt & Giribet, 2006).**

Diagnostic genetic markers from 486 aligned nucleotide sequences of mitochondrial 16S ribosomal DNA were developed for the four closely related species of dreissenoid and corbiculoid bivalves that have invaded North America **(Stepien *et al*., 1999)**. The sequences were compared to 16SrDNA secondary structure models for drosophila and *Mytilus edulis* and *Pecten maximus* to identify stem (paired) and loop (unpaired) regions.

A single–colour in Fluorescence In Situ Hybridization (FISH) of spermatocyte chromosomes of the periwinkle *Melarhaphe neritoides* (Caenogastropoda) was used to map repeated units of the two rDNA families (18S, 28SrDNA and 5SrDNA) and to test the presence of (GATA) and (TTAGGG) repeats in the genome of this species **(Colomba *et al.*, 2002)**.

Partial 18S rRNA gene sequences of four macrodasyid and one chaetonotid gastrotrichs were obtained and compared with the available sequences of other gastrotrich species and representatives of various metazoan phyla **(Manylov *et al*., 2004 and Zrzavy, 2003).**

Many different DMRT genes (family of genes are considered to be involved in sex determination or differentiation) sequences from different species of small fresh water teleosts, medaka *(Oryzias latipes)* and the platyfish (*Xiphophorus maculatus*) were analysed to compare different species **(Kondo *et al*., 2002)**.

Squeaker catfishes (pisces, Mochokidae, *Synodontis*) are widely distributed throughout Africa and inhabit a biogeographic rang similar to that of the exceptionally diverse cichlid fishes. The existence of six major lineages of *Synodontis* in East Africa was revealed **(Koblmüller *et al*., 2006)**. The six lineages showed a clear phylogeographic pattering. Also, an accelerated rate of molecular evolution in *Syndodontis*, which might be the consequence of co-evolutionary dynamics was detected.

Although, the actinopterygii sarcopterygii hypothesis become gradually accepted by most morphological phylogeneticists, opinions nevertheless differed withen the Dipnoi or the Actinistia as the sister

groups of the tetrapods **(Xia *et al*., 2003)**. **Meyer and Wilson (1990)** addressed this question in a molecular study that was based on portions of two mitochondrial (mt) genes, Cytochrome *b* and 12S rRNA. The taxon sampling included the coelacanth, a lungfish and a frog, plus a teleost that was used to root the tree. **Arnason *et al*. (2004)** described basal relationships among gnathostome vertebrates as reconstructed by analysis of complete mitochondrial DNA sequences. The study included all major extant groups of both tetrapods and fishes to root the trees. Similarly, analysis of 18S and 28SrRNA genes (both nuclear) do not support the commonly accepted tree.

The 5S ribosomal DNA (5SrDNA) of higher eukaryotes is organized in repeat units of tandem arrays composed of a 5SrDNA coding region, conserved even among non- related taxa, and a variable Non- Transcribed Spacer (NTS) sequence. To contribute knowledge on the organization and evolution of vertebrate 5SrDNA, **De Rosa Santos *et al.* (2006)** used PCR nucleotide sequencing, Southern blot hybridization and chromosome FISH to investigate 5SrDNA tandem repeats in the South American Curimatidae fish. The chromosomal stability of this family means that, it is often difficult to identify species-specific cytogenetic markers. Although recent and more discriminating molecular techniques such as DNA sequencing and Southern blotting after new approaches to the identification of species- or population-specific markers which can contribute to understanding evolution in the Curimatidae.

The interrelationship of three major extant lineages of vertebrates (Hagfishes, Lampreys and Gnathostomes) is considered to be a particularly important issue in evolution. So, nearly complete rDNA sequences from each of these major lineages were compared. For this comparison, complete 28SrDNA sequences were obtained from the lancelet, the hagfish, the lampreys and cartilaginous fishes and were than analyzed with previously reported 25S and 18S rDNA sequences from other chordates. All these analyses provide very strong support for the monophyly of the cyclostomes, and prove that the hagfish has the longest 28SrRNA gene known in any organism **(Mallatt & Sullivan, 1998).**

Nuclear 18S rRNA gene sequences were determined during a phylogenetic study of the evolutionary relationships of North American sturgeon (Acipenseridae) paddlefish *(Polyodon spathula)*. In the course of **Krieger and Fuerst (2004)** study, intra-individual variations of the 18S rRNA genes were discovered in all nine species of sturgeon examined, while no variation was detected for the gene in the North American paddlefish. The discovery of multiple sequence variants of the 18S rRNA gene in an individual is unexpected; although most eukaryotes posses many copies of the rDNA unit in their genomes, the copies are normally thought to be homogenized within an individual as well as in a species by concerted evolution. Also, the presence of intra-individual variations and the maintenance of multiple 18S rDNA sequence variants in sturgeon may be related to the polyploidy status of the sturgeon genome. So, the population characteristics of sturgeon were evaluated by **Kenndy *et al*. (2007)** and **Welsh *et al*. (2008).**

DNA sequences of 18S rRNA gene and Random Amplified Polymorphic DNA (RAPD) were compared to discriminate the endangered Manchurian trout from the rainbow trout *Oncorhynchus mykiss* and amago salmon *Oncorhynchus masou ishikawai* that also live in the same fresh water region **(Jin *et al.*, 2006)**. The partial 18S rRNA gene sequences analysis (667 bp) showed conserved sequences except a few unique substitutions among the three fresh water salmonids. Also, DNA–level variations were detected among different salmon species **(Beacham *et al*., 2005 & 2008; Flannery *et al*., 2007 and Baumsteiger & Kerby, 2009).**

Nearly complete sequences of rRNA genes (28S and 18S rRNA) can be generated more easily, from a wide range of deterostomes. At over 5000 nt. in length, the rRNA gene family is known to contain phylogenetic information at many taxonomic levels. **Mallatt and Winchell (2007)** sequenced 28S and 18S rRNA genes of seven deuterostomes (brittle star *Ophiomyxa*, lizard *Anolis*, turtle *Chrysemy*s, sixgill shark *Haxanchus*, electric ray *Narcine*, southern Hemisphere Lamprey *Geotria* and Atlantic hagfish *Myxine*) and investigated the phylogenetic relationship of different species.

Mapping of nuclear 18S rRNA and 5SrRNA genes and distribution of constitutive heterochromatin supposedly AT-rich were characterized on two isolate populations of *Salminus brasiliensis*, the biggest characid fish (characiformes) and three population of *Salminus hilarii*. The position of 18S rDNA cluster ideutified by FISH coincide with Chromomycin A_3 labeling (CMA^+) in the long arm telomeric portion of sixth pair **(Souza *et al.*, 2008).**

Much was already known about tilapia genomes at the molecular level. The genome size of several species has been measured at around 1pg **(Majumdar & McAndrew, 1986)**. **Mansour *et al*. (1998)** report the complete tilapia (*O. niloticus*) insulin gene sequence which has three exons (one untranslated), two intron distribution found in all insulin genes sequenced.

The regulatory sequence including proximal promoter, untranslated exon 1 and intron 1 of the β-actin gene from tilapia (*O. niloticus*) has been isolated and spliced to a β-galactosidase reporter gene to test its activity and to compare with carp β-actingene **(Hwang *et al.*, 2002)**. Also, the nucleic acid and deduced amino acid sequences of Insulin-like Growth Factor-1(IGF-1) cDNA have been reported for *O. niloticus* **(Vera Cruz *et al.*, 2006)**.

From a comparative point of view, the regulatory elements of chinook salmon, tilapia and rainbow trout prolaction genes have been isolated and sequenced **(Argenton *et al*., 1996 and Poncelet *et al.*, 1996)**. Also, the study of prolaction gene in *Sparus auratus*, correlates well with those published on tilapia and rainbow trout **(Astola *et al.*, 2003)**.

The ghrelin stimulated Growth Hormone (GH) and cDNA encoding precursor protein from the stomach of euryhaline tilapia, *O. mossambicus* and the sequence of 20 amino acid tilapia ghrelin have been identified by **Kaiya *et al*. (2003)**. The complementary and genomic DNA sequences of ghrelin (28 amino acid growth hormone-releasing peptide) were identified in *Tilapia* species (*O. niloticus*) **(Parthar *et al*., 2003)**.

Neuropeptid Y (growth promoting action): a 36 amino acid sequence abundantly expressed in red tilapia (*Oreochromis* sp). **Carpio *et al*. (2006)** cloned and reported the cDNA sequence coding for tilapia 36 amino acid neuropeptide Y.

The full- length sequence of tilapia (*O. mossambicus*) Follicle Stimulating Hormone β (FSHβ) gene was determined **(Rosenfeld *et al.,* 2001)**, then the sequencing analysis of the gene coding for FSHβ subunit α compared between tilapia (*O. mossambicus*) and gold-fish (*Carassius auratus*). Also, Rapid Amplification of cDNA Ends-PCR (RACE-PCR) and sequencing techniques was used to determine the full sequence encoding the nature peptide and the 3′ untranslated region of the glycoprotein α subunit of tilapia (*O. mossombicus*) **(Gur *et al*., 2001)**.

The complete serine 8- type Gonadotropin Releasing Hormone (GnRH) coding sequence with a substantial 5′ regulatory sequence has been isolated and characterized in chicken **(Carolsfeld *et al.,* 2000)** and in Nile tilapia (*O. niloticus*) from a relevant genomic library **(Farahmand *et al.,* 2003)**.

The amino acid sequence reduced from 5′ truncated cDNA encoding estrogen receptor was investigated for different fish species Atlantic croaker (*Micropogonias undulates*), tilapia (*O. niloticus*), rainbow trout and catfish (*Ictalurus punctatus*) **(Kim *et al*., 2002)**.

The stearoyl-CoA desaturase cDNA in tilapia (*O. mossambicus*) was cloned by Real Time-PCR (RT-PCR), and it was compared with

those in grass carp, common carp and milkfish. Nucleotide sequence analysis revealed that the full length of cDNA (1172 bp) clone encompasses 1008 bp Open Reading Frame (ORF) encoding 336 amino acid residues. The stearoyl-CoA desaturase nucleotide sequence analysis was compared between different species **(Hsieh *et al*., 2004)**.

A comparative analysis of the Fox 12 (a putative transcription factor involved in ovarian development and function) sequences of 10 vertebrate species, including 7 from mammals and 3 from fish (including *O. niloticus*), revealed that the entire (ORF) is under purifying selection leading to strong protein conservation **(Wang *et al.*, 2004)**.

Expressed Sequence Tag (EST) cataloging and profiling can provide the basis for functional genomic research. The analyses of ESTs provide significant additional functional, structural, and evolutionary information **(Quackenbush *et al.*, 2000 and Bouck & Vision, 2007)**. The identification of genes expressed in the cells of hypothalamus will be an important step to understand the gene function and its physiological functions. EST strategy is remarkably suitable for the large-scale screening for gene expression profile analysis in addition to gene identification. EST libraries of various organs and tissues have been constructed for zebrafish **(Winkler & Moon, 2001)**, channel catfishes (*Ictalurus punctatus*) **(Ju *et al.*, 2000)**, tilapia (*O. niloticus*) **(Hamilton *et al.*, 2000)** and tilapia (*O. mossambicus*) **(Shiue *et al.*, 2004 and Chu *et al*., 2006)**.

Prodynorphin cDNAs were cloned from the brain of the eel *Anguilla rostrata* and the Nile tilapia (*O. niloticus*). These teleost prodynorphin sequences have distinct α-neoendorphin, dynorphin A and dynorphin B sequences and a novel opioid sequence. The relationship of these teleost prodynorphin sequences to other actinopterygian and sarcopterygian prodynorphin sequences was discussed by **Alrubaian *et al.* (2006)**. The phylogenetic analysis clearly aligned the tilapia Dopamine Receptor (DA-R) with other vertebrates and showed high similarity to DA-Rs of mullet, fugu, xenopus, mouse and turkey **(Levavi-Sivan *et al.*, 2005)**.

Nucleotide and amino acid sequences of the tilapia (*O. mossambicus*) Melanocortin-1 Receptor (MC1R) which regulates the skin darkness invertebrates were elucidated by **Van der Salm *et al.* (2005)**. The sequencing analysis was compared according to adaptation to three different backgrounds (black, white and grey back ground tanks).

The r-protein L18 in concert with L5 and L25 interacts with the 5SrRNA, which is a component of the large ribosomal subunit. A few genomic sequences have been reported in higher eukaryotes; Xenopus **(Beccari & Mazzetti, 1987)** and human **(Puder *et al.*, 1993)**. Also, **Molina *et al.* (2001)** reported the molecular cloning of tilapia (*O. niloticus* & *O. mossambicus*) L18 ribosomal protein gene and its nucleotide sequence.

The stock identification and the evolutionary relationships of tilapiine fishes of the genera *Oreochromis, Sarotherodon* and *Tilapia* (Pisces: Cichidae) were detected using allozyme analysis and

Restriction Fragment Length Polymorphism (RFLP) analysis of mitochondrial DNA **(Seyoum, 1989 & 1990; Seyoum & Kornfield, 1992 and El-Serafy *et al*., 2007)** and using RAPD markers **(Ahmed *et al*., 2004).** Also, **El Serafy *et al.* (2003 & 2007)** used RFLP analysis of nuclear 18S rRNA gene to identify and detect the evolutionary relationships of *Tilapia* species.

Agnese *et al*. (1997) reported that, the genetic differentiation among 17 natural populations of the Nile tilapia *O. niloticus* -in Africa- was analyzed using RFLP of mitochondrial DNA (mtDNA). It was found that sixteen variable nuclear loci showed that these populations could be clustered in four groups; West African populations, Ethiopian, Rift valley populations and Nile drainage (including Manzalla, Cairo and Lake Edward).

African cichlid fishes are composed of two major linages, the haplochromines and the tilapiines. Whereas, the phylogenetic relationships of the haplochromines have been studied extensively, molecular phylogenetic analysis of tilapiine fishes was undertaken to know more about the relationships among the tilapiine species **(Nagi *et al*., 2001)**. A segment of mitochondrial DNA encompassing the terminal part of the tRNA gene and the most variable part of the control region was amplified by PCR with DNA samples isolated from 42 tilapiine species, and the amplification products were subjected to heteroduplex analysis and sequencing. Phylogenetic trees based on 68 sequences revealed the existence of 11 sequence groups and 11 single- sequence branches.

The cichlid subfamily Cichlasomatinae has one of the most complex taxonomic histories among cichlid groups. To recover the relationships among Cichlasomatinae genera and to test their monophyly, **Musilová *et al.* (2008)** analyzed sequences from two mitochondrial (16SrRNA & Cytochrome *b*) and one nuclear marker (first intron of 70S ribosomal gene) totaling 2236 bp. The data suggested that all genera of Cichlasomatinae except *Aequidens* are monophyletic.

The cloning and nucleotide sequence of PCR-generated 5SrDNA from the tilapiine cichild fish (*O. niloticus*) was reported. Two types of 5SrDNA were detected by insertions and base substitutions within the NTS. Two 5SrDNA loci were observed by FISH technique in metaphase spreads of tilapia chromosome. FISH using 18S rDNA probe and silver nitrate sequential staining of 5S-FISH slides showed three 18S rDNA loci that are not syntenic to the 5SrDNA loci **(Martins *et al.*, 2000).**

Studies on rRNA genes have gained prominence in abroad rang of animals and plants, especially in relation to species or population characterization and evolutionary relationships. In higher eukaryotes, tandem arrays of rRNA genes are organized in two distinct multigene families composed of multiple of copies. The major class of rDNA comprises the genes that code for the 18S, 5.8S and 28SrRNAs, which are related to the Nucleolar Organizer Regions (NOR2). The general nucleotide sequence and positions of these two multigene families have contributed to understanding the structure, organization and evolution of genomes (**Martins *et al.*, 2002)**. So, they cloned 5SrDNA

copies from the tilapia fish, *O. niloticus*, and determined the nucleotide sequences of the coding region and of the NTS.

The size of the cichlid genome has been estimated from 30 measurements of 23 species **(Gregory, 2001**). The mean size 1.1pg has independent measurements of *O. niloticus* average 1.08pg. **Kocher, (2004)** predict that a haploid Nile tilapia genome is 1.06 x 10^9 base pairs. The base composition of the Nile tilapia genome is slightly AT-baised. An analysis of 58kb of coding sequences from *O.niloticus* shows A: C: G: T in the proportions 0.27: 0.23 : 0.23: 0.26 sequence of 100kb cosmid containing a Hox cluster **(Santini *et al*., 2003)**, as well as three Bacterial Artificial Chromosomes (BACs) containing Opsin gene clusters shows the proportion 0.29: 0.21: 0.21: 0.29.

The major randomly repeated DNAs of the Nile tilapia are well–studied and have been mapped by FISH technique **(Franck *et al*., 1994).** Satellite DNA Sequence A (SATA) is a ~ 230 bp sequence present in ~ 105 copies and found in the centromeric regions of all chromosomes. Satellite DNA Sequence B (SATB) is a ~ 1900 bp sequence found primarily on the short arm of chromosome 4. 18S rDNA sequences are found on chromosomes 8, 10 and 15.5SrDNA repeats are found on chromosomes 3, 9 and 13. Cichlids have a standard telomeric repeat (TTAGGG) which is also found interstitially on chromosome 1, possibly indicating recent chromosome fusions **(Martins *et al*., 2004).**

The goal of the present study is to investigate some new bases for identification, characterization and phylogeny of *Tilapia* species; *O. niloticus*, *O. aureus*, *S. galilaeus* and *T. zillii*, inhabiting the River Nile.

Though, scarce work has been done on fish scales in direction of Atomic Force Microscopy (AFM), therefore, this work is directed to use multimode AFM to investigate the surface morphology of the scales of four *Tilapia* species. In addition, this technique is used to differentiate the four species based on the scanning over rostral rims, radial grooves, focus regions and posterior parts of the scales. Also, AFM images of the inter-radial denticles are obtained and analyzed using SPIP program to differentiate the four studied species.

Also, this work is directed to use X-ray photographs to describe the endoskeleton of different *Tilapia* species. As well as, the types and arrangements of both lower jaw teeth and lower pharyngeal teeth of the studied species are investigated to illustrate their taxonomic variations.

Furthermore, the present study is also extended to use one of the more recent molecular biological techniques, which is the sequencing technique. The PCR amplified 18S rRNA gene is sequenced to detect the nucleotide sequence and nucleotide substitution of the gene for each *Tilapia* species.

Using the bioinformatics programs to analyze the sequencing data, provide the phylogeny tree, RNA secondary structure and amino acid sequence to emphasize on the nature of intra- and inter-specific differences among *Tilapia* species.