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# Influence of Geographical Isolation on Genetic Diversity of Redbelly Tilapia, *Coptodon zillii* Gervais, 1848 and Microhabitat Transformation Modulated by Gill Ectoparasitic Helminthes

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**ABSTRACT**

The study investigated the genetic diversity of the redbelly tilapia (*Coptodon zillii*) from three Egyptian aquatic ecosystems; Rayyan Lake, Burullus Lake, and the River Nile with the microhabitat utilization patterns of three gill parasites (monogenea, digenea, and copepoda). Results rejected the null hypothesis that geographical isolation does not affect genetic diversity, revealing distinct evolutionary divergence among the populations. Phylogenetic analysis showed strong bootstrap support (94-100%), dividing the Egyptian isolates into two sub-populations: one with Burullus Lake and the Nile River isolates and another with the Rayyan Lake isolate evolving separately. Additionally, the study dismissed the assumption that microorganisms exhibit identical microhabitat usage. Each parasite taxon displayed unique attachment, distribution, and feeding strategies, leading to specific microhabitat transformations. Digenean *Centrocestis formosanus* formed cysts within gill cartilage, monogeneans lodged between gill lamellae, and copepods (*Ergasillus sieboldi*) gripped filaments using specialized antennae. These adaptations suggest resource partitioning facilitates coexistence in shared environments. The findings highlight how ecological and evolutionary factors shape genetic divergence in the redbelly tilapia and parasite niche specialization. The discussion explores potential drivers of clustering patterns and evolutionary relationships among tilapia isolates, as well as strategies for microhabitat utilization. Overall, the study underscores the role of environmental heterogeneity in influencing both host genetics and parasite ecological dynamics.

## INTRODUCTION

Cichlids have successfully adapted to a diverse range of aquatic environments across their distribution area, including brackish waters, small streams, large rivers, small crater lakes, and some of the largest lakes on Earth. The redbelly tilapia, *Tilapia zillii* is native to Africa, belongs to family Cichlidae (Teleostei, Perciformes) and is regarded as one of the most widely distributed bony fish worldwide **(Trewavas, 1982; Pillay, 1990; Salzburger, 2009; Dunz & Schliewen, 2013; Teimori *et al*., 2017; Gu *et al*., 2018)**. They are also found in extreme habitats, such as the alkaline lakes of East Africa. Approximately, fifty percent of the currently recognized diversity of cichlid species has emerged from

remarkable adaptive radiations occurring in three of Africa's Great Lakes: Tanganyika, Malawi, and Victoria **(Salzburger *et al*., 2014)**.

Due to their extraordinary phenotypic variation and high rates of speciation, the cichlid characterization in these lakes have attracted significant attention within the field of cichlid research **(Kocher, 2004; Turner, 2007; Seehausen *et al*., 2008; Santos & Salzburger, 2012; Seehausen, 2015; Salzburger, 2018)**. The Cichlidae family of fishes stands out as the most species-rich family of vertebrates, with over 3,000 species found across Central and South America, Africa, Madagascar, and southern India. Cichlids exhibit remarkable diversity in each of these regions and have consistently demonstrated a propensity for rapid radiation and sympatric speciation **(Schliewen *et al*., 1994; McKaye *et al*., 2002)**. Notably, the swift radiation of cichlid species in the Great Lakes of East Africa has garnered significant attention, as nearly 2,000 species have evolved in this relatively recent evolutionary timeframe. These species represent an extraordinary reservoir of phenotypic variation, akin to a natural laboratory.

The genetic diversity of different tilapia species in aquatic ecosystems of Egypt has been studied by few numbers of authors **(Hassanien & Gilbey, 2005; El-Serafy *et al.*, 2006; Abdel-Kader *et al*., 2013; El-Fadl *et al*., 2016; Soliman *et al*., 2017)**. Tilapiïne fish have been studied at the genetic level to clarify the population genetic structure, genetic diversity, taxonomy and species identification and these data have proven helpful in fish farming and fisheries management **(Soliman *et al*., 2017; Kwikiriza *et al.*, 2024)**. **Soliman *et al*. (2017)** accomplished a comparative analysis on the population genetic structure of the redbelly tilapia inhabiting Lake Nasser (freshwater habitat), Lake Idku (brackish water habitat) and Max Bay (marine habitat). These authors employed three mtDNA markers, namely COI, D- loop, and CYTB, and demonstrated a clear genetic variation in the structure of the population of *T. zillii* from the three fragmented ecosystems. They correlated the genetic variation to physical barriers such as Aswan High Dam at Lake Nasser, habitat isolation at Idku Lake and limited population size of *T. zillii* at Max Bay that is inhabited by fish escaping from the nearby ponds and fish farming facilities. Moreover, low genetic diversity of the redbelly tilapiine fish at Max Bay was correlated by **Soliman *et al*. (2017)** to chemical barriers where the bay exhibits higher salinity level than other habitats. Under these habitat circumstances, a limited subset of the genotypes of the redbelly tilapia has been adapted to survive salt-rich habitats. At Lake Idku, habitat size was correlated to the genetic diversity of the redbelly; at Idku Lake, there was a rapid change in the demographic aspects as the lake lost about 390km² since 1800. Habitat-related variation in the genetic diversity was reported by **Hassanien and Gilbey (2005)** in the Nile tilapia, *Oreochromis niloticus* and by **Szitenberg *et al*. (2012)** in the redbelly tilapia inhabiting the Dead Sea system.

The null hypothesis assumes that geographical isolation and habitat fragmentation show no influence on the genetic diversity of the redbelly tilapia irrespective of the

hydrological and geological features of the hospitable ecosystems. In view of this postulation, it is expected that the population of the redbelly tilapia resident in Rayyan Lake (closed inland, spatially isolated habitat) possesses similar genetic characteristics to its counterparts in the River Nile and Burullus Lake (open aquatic habitats). This null hypothesis was tested in the present study by conducting a comparative analysis of the genetic characters of the redbelly tilapia (phylogenetic tree and genomic buildup).

The null hypothesis also assumes that living microorganisms show identical microhabitat utilization patterns, in terms of food, space and reproduction. This null hypothesis was tested in the present study by monitoring some *in situ* maneuvers practiced by three parasitic taxa, namely Monogenea (*Cichlidogyrus* Paperna, 1960, *Scutogyrus* Pariselle and Euzet, 1995, *Gyrodactylus* von Nordmann, 1832, *Macrogyrodactylus* Malmberg, 1957), Digenea (*Centrocestis formosanus* Nishigori, 1924) and Copepoda (*Ergasillius sieboldi* von Nordmann, 1832) from the gills of the redbelly tilapia from closed and open aquatic ecosystems.

## MATERIALS AND METHODS

### 1. Aquatic ecosystems under investigation

The studied ecosystems are located at the Nile Delta and close area, Egypt (Fig. 1A). Three differing–water quality habitats were defined, namely Rayyan Lake (Fig. 1B) (coordinates: 29°34'22"N 030°35'23"E), Burullus Lake (Fig. 1C) (coordinates: 31°29′N 30°52′E) and Damietta Branch of the River Nile (Fig. 1D) (coordinates: 31°03′00″N 31°23′00″E).

Tilapia muscle piece was fixed in 96% ethyl alcohol and proceeded for DNA extraction and amplification with PCR using specific primers (COX1 gene) according to **Mendoza-Palmero *et al*. (2015)**. The coding regions were used to determine the phylogenetic relationship between phyla while the non-coding regions are used to study the relationships among closely related genera congeneric species **(Nolan & Cribb, 2005)**.

### 2. DNA extraction

Approximately 20–100 mg of muscle tissue was excised using a sterile scalpel and forceps. The tissue was homogenized in 600µl of extraction buffer, and the resulting paste was transferred into a 1.5ml microcentrifuge tube (MCT). The sample was incubated in a water bath at 37°C for one hour, followed by an additional incubation at 55°C for one hour.

Following incubation, the mixture was centrifuged at 5000 rpm for 10 minutes. The supernatant was carefully transferred to a new MCT, to which an equal volume of Phenol:Chloroform:Isoamyl alcohol (25:24:1) was added and mixed thoroughly. The

sample was centrifuged at 12000 rpm for 10 minutes, and the upper aqueous phase was transferred to a fresh MCT, taking care to avoid the interphase.

Subsequently, Chloroform:Isoamyl alcohol (24:1) was added in equal volume to the aqueous phase, mixed thoroughly, and centrifuged at 12000 rpm for another 10 minutes. The upper aqueous layer was again transferred to a new MCT. To precipitate DNA, 0.1 volume of 3M sodium acetate and an equal volume of ice-cold 100% ethanol were added. The solution was mixed until DNA clumps or pellets were visible and then incubated at -20°C for one hour.


**Fig. 1A.** Hand drawn map of the Nile Delta, Egypt showing: Burullus Lake (yellow solid circle) and Damietta Branch of the River Nile in the vicinity of Mansoura (red solid circle). Note that the bright green color in the map reflects the fertility of this magic landscape (Nile Delta), however yellow color reflects the eastern and western desert around Nile Delta. Note the directions according to the compass.

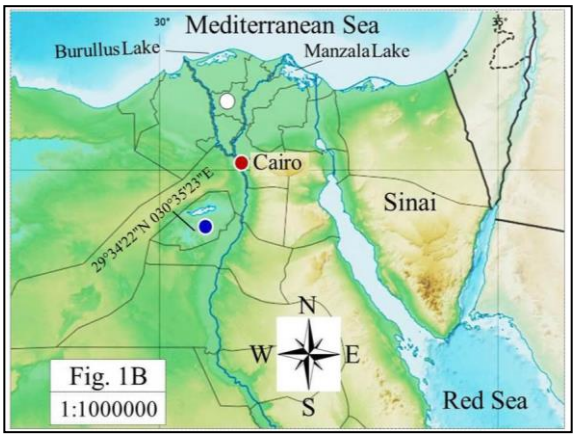


**Fig. 1B.** Geologic map of the explored ecosystems. The blue solid circle denotes Rayyan Lake at the south-western region of Cairo. The white solid circle denotes the Nile Delta. Note the directions according to the compass.


**Fig. 1C.** Google Earth map showing Burullus Lake in close proximity to the Mediterranean Sea. Note the directions according to the compass. Note also the sampling locality at Baltim district (red sold circle). Scale bar = 5 km.

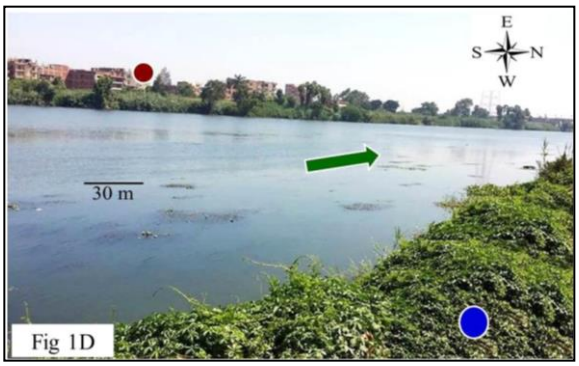


**Fig. 1D.** Mobile photograph showing a sector of Damietta Branch of the River Nile at Mansoura. Note the dense vegetation cover on the banks (blue solid circle). Note the directions according to the compass. Note also a human encroachment on the River Nile: suburban settlements (red solid circle). Scale bar = 30 m.

### 3. Histological preparations

Following a 48-hour immersion in a 10% formaldehyde fixative, the first gill arch from a medium-sized redbelly tilapia (*Tilapia zillii*) collected from each habitat was sent to the Histopathology Laboratory for processing and staining with hematoxylin and eosin. The gill tissues were sliced into 5µm sections along a plane parallel to the long axis of the filaments. Each section was then stained using either the standard H and E method or PAS stain to elucidate the histological differences in the gills of the cichlid fish across the three habitats studied.

The PAS stain is a histochemical technique in which periodic acid oxidizes carbon to carbon bonds, resulting in the formation of aldehydes. These aldehydes subsequently react with fuchsin-sulfurous acid, producing a magenta color. A high-quality Schiff reagent will quickly develop a red-purple color, while a degraded Schiff reagent will exhibit a delayed response, yielding a deep blue-purple shade. The periodic acid–Schiff stain is effective in highlighting specific polysaccharides, particularly glycogen and mucoproteins. The tissues were preserved in Bouin fixative for 24 hours before being transferred to 70% alcohol. Subsequently, they underwent dehydration through a series of graded ethanol solutions (70%, 80%, 90%, and 100%) and xylene, followed by embedding in paraffin. Using a rotary microtome, 5-µm sections were sliced from the paraffin blocks and allowed to dry overnight at 37°C. After deparaffinization, the sections were stained with xylene. For the histochemical identification of neutral mucin, the tissue sections were treated with periodic acid/Schiff (PAS) and aldehyde fuchsin (AF) staining. To identify sulfated glycoconjugates, a histological technique was employed **(Kumari *et al.*, 2009)**.

Glycoproteins containing carboxyl groups, O-sulfate esters of glycoproteins, and highly sulfated glycoproteins in the gill mucous cells were stained with alcian blue (AB) at pH levels of 2.5, 1, and 0.5, respectively **(Díaz *et al.*, 2010)**. The stained sections were then mounted with Entellan and examined under a light microscope (Leica DMI 6000B), with images captured using a Leica DFC 490 camera (Leica Microsystems).

## RESULTS

### 1. Molecular biological studies on *Tilapia zillii* (*Coptodon zillii*)

Ten studied isolates from *Tilapia zillii* (*Coptodon zillii)* collected from three habitats in Egypt (Rayyan Lake at Fayoum Governorate, Burullus Lake at Kafr El-Sheikh Governorate, and Damietta Branch of the River Nile at Dakahlia Governorate) were compared to relatives around the globe, and identified on molecular basis using mitochondrial DNA region (Cytochrome C Oxidase Subunit I (COX1) gene). From Blast analysis (Tables 1-3), studied isolates displayed 100% - 99.85% similarity with the formerly identified isolates of *Tilapia zillii* on the Genbank. Figs. (2-4) represent the constructed phylogenetic tree of the studied two isolates and their relatives which comes in line with the previous morphological identification. The GenBank accession numbers and sequences of the studied isolates are documented in Table (4).

#### 1.1. *Tilapia zillii* (*Coptodon zillii*) isolate TZ77 collected from Wadi El-Rayan at Fayoum Governorate (Egypt)

The sequence length, AT and GC content for studied taxa are recorded in Table (4). The sequence length was 751 bp. AT% content was 49.8 % while GC% content was 50.2 %. The BLAST search showed a pairwise identity (PI) of 100 % with *Tilapia zillii* voucher BNF 213 collected from Southeastern Nigeria and identity (PI) of 99.85 % for seven *Tilapia zillii* isolates from Pakistan, Philippines and Nigeria (Table 1).

The phylogenetic analysis for *Tilapia zillii* (isolate TZ77) collected from Rayyan Lake at Fayoum Governorate using the COX 1 gene is presented in Fig. (2). The phylogenetic tree showed two main clades. The first clade includes *Tilapia zillii* (isolate TZ77) (Egypt) and *Tilapia zillii* voucher BNF 213 (Southeastern Nigeria) with bootstrap support (99% BS). The second clade includes other seven *Coptodon zillii* isolates with bootstrap support (100 % BS).

**Table 1.** Comparison of the similarity percentage of *Tilapia zillii* (*Coptodon zillii)* collected from Rayyan Lake at Fayoum Governorate (Egypt) with the other *Tilapia zillii* isolates previously registered in NCBI

| Taxa | Country | The most similar sequences in Gene Bank database | |
|---|---|---|---|
| | | GenBank Accession Number | Identity |
| *Coptodon zillii isolate* TZ77 | Egypt (Wadi El-Rayan at Fayoum Governorate) | PP097389 | 100 % |
| *Tilapia zillii voucher BNF 213* | Southeastern Nigeria | HM882888 | 100 % |
| *Coptodon zillii voucher BLF-CZ9* | Philippines | MG407386 | 99.85 % |
| *Coptodon zillii voucher NGA-2014-0590* | Nigeria | ON072305 | 99.85 % |
| *Coptodon zillii voucher NGA-2014-0203* | Nigeria | ON072277 | 99.85 % |
| *Coptodon zillii voucher BLF-CZ4* | Philippines | MG407385 | 99.85 % |
| *Coptodon zillii voucher NGA-2014-0346* | Nigeria | ON072399 | 99.85 % |
| *Coptodon zillii voucher NGA-2014-0170* | Nigeria | ON072344 | 99.85 % |
| *Coptodon zillii voucher NGA-2014-0553* | Nigeria | ON072304 | 99.85 % |

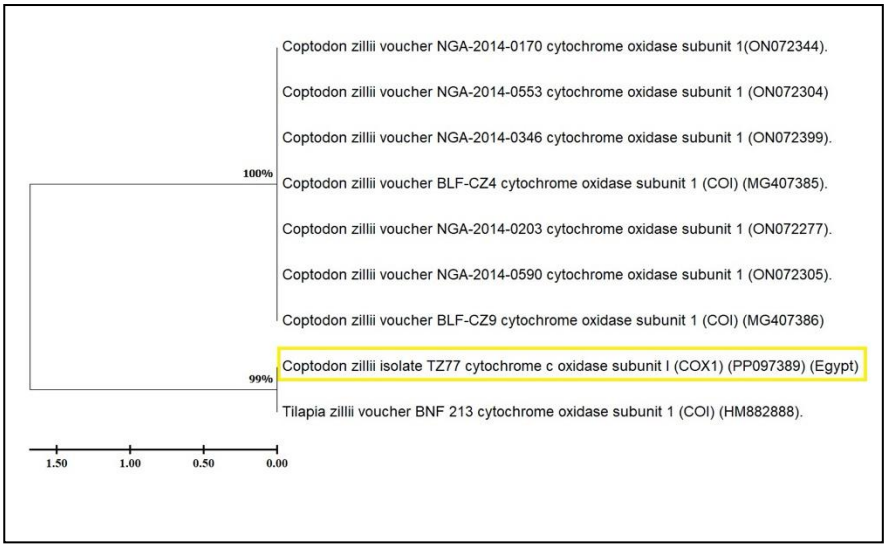


**Fig. 2.** Phylogenetic tree for *Tilapia zillii (Coptodon zillii)* isolate TZ77 collected from Wadi El-Rayan at Fayoum Governorate – Egypt inferred from COX 1 gene sequences obtained from Gene Bank (highlighted in yellow color). Bootstrap tests were performed with 1000 replications

### *1.2. Coptodon zillii isolate Cz85 collected from Burullus Lake at Kafr El-Sheikh Governorate (Egypt)*

The sequence length, AT and GC content for studied taxa are recorded in Table (4). The sequence length was 619 bp. AT% content also was 49.6 % while GC% content was 50.4 %. The BLAST search showed a pairwise identity (PI) of 100 % for seven *Coptodon zillii* isolates collected from Pakistan, Egypt and UK and pairwise identity (PI) of 99.84 % with *Coptodon zillii* voucher Fish-CZ-4 collected from Pakistan (Table 2).

The phylogenetic analysis for *Coptodon zillii* isolate Cz85 collected from Burullus Lake at Kafr El-Sheikh Governorate using the COX 1 gene is presented in Fig. (3). The phylogenetic tree showed two main clades. The first clade is divided into two sub-clades; first sub-clade includes *Coptodon zillii* isolate Cz85 (Egypt) with with bootstrap support (94 % BS) and the second sub-clade includes six *Coptodon zillii* isolates with bootstrap support (96% BS). The second clade includes two *Coptodon zillii* isolates with bootstrap support (100 % BS). The second clade includes two isolates from *Coptodon zillii* (*Coptodon zillii* voucher MC_38 & *Coptodon zillii* isolate 72iv) from United Kingdom and Pakistan with bootstrap support (95 % BS).

**Table 2.** Comparison of the similarity percentage of *Coptodon zillii* isolate Cz85 collected from Burullus Lake at Kafr El-Sheikh Governorate (Egypt) with the other *Coptodon zillii* isolates previously registered in NCBI

| Taxa | Country | The most similar sequences in GeneBank database | |
|---|---|---|---|
| | | GenBank Accession Number | Identity |
| *Coptodon zillii isolate Cz85* | Egypt (Burullus Lake at Kafr El-Sheikh Governorate) | PP097446 | 100 % |
| *Coptodon zillii isolate 72ii* | Pakistan | OQ330936 | 100 % |
| *Coptodon zillii voucher MC_38* | United Kingdom | KM438549 | 100 % |
| *Coptodon zillii isolate 72iv* | Pakistan | OQ330941 | 100 % |
| *Coptodon zillii isolate 72iii* | Pakistan | OQ330939 | 100 % |
| *Coptodon zillii haplotype 7* | Egypt | KY465481 | 100 % |
| *Coptodon zillii isolate D* | Pakistan | MT707447 | 100 % |
| *Coptodon zillii haplotype 3* | Egypt | KY465477 | 100 % |
| *Coptodon zillii voucher Fish-CZ-4* | Pakistan | MN368907 | 99.84 % |

### *1.3. Coptodon zillii isolate TZ80 collected from Damietta branch of the River Nile at Dakahlia Governorate (Egypt)*

The sequence length, AT and GC content for studied taxa are recorded in Table (4). The sequence length was 619bp. AT% content was 49.4% while GC% content was 50.6 %. The BLAST search showed a pairwise identity (PI) of 99.78 % for eight *Coptodon zillii* isolates from Pakistan, Mauritania and France (Table 3).

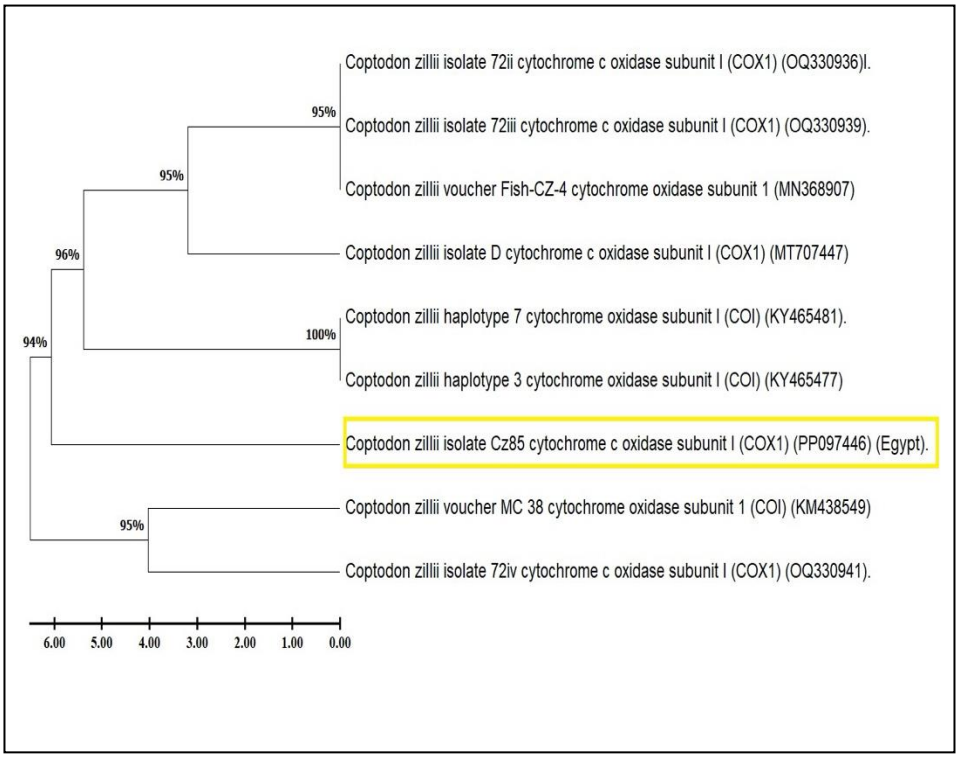


**Fig. 3.** Phylogenetic tree for *Coptodon zillii* isolate Cz85 collected from Burullus Lake at Kafr El-Sheikh Governorate – Egypt inferred from COX 1 gene sequences obtained from Gene Bank (highlighted in yellow color). Bootstrap tests were performed with 1000 replications

**Table 3.** Comparison of the similarity percentage of *Coptodon zillii* isolate TZ80 collected from Damietta branch of the River Nile at Dakahlia Governorate (Egypt) with the other *Coptodon zillii* isolates previously registered in NCBI

| Taxa | Country | The most similar sequences in GeneBank database | |
|---|---|---|---|
| | | GenBank Accession Number | Identity |
| *Coptodon zillii isolate TZ80* | Egypt (Damietta branch of the River Nile at Dakahlia Governorate) | PP097456 | 100 % |
| *Coptodon zillii voucher Fish-CZ-4* | Pakistan | MN368907 | 100 % |
| *Coptodon zillii voucher Fish-CZ-6* | Pakistan | MN368909 | 100 % |
| *Coptodon zillii strain N2* | France | KJ938220 | 100 % |
| *Coptodon zillii voucher Fish-CZ-7* | Pakistan | MN368910 | 100 % |
| *Coptodon zillii voucher Fish-CZ-10* | Pakistan | MN368913 | 100 % |
| *Coptodon zillii voucher Fish-CZ-2* | Pakistan | MN368905 | 100 % |
| *Coptodon zillii strain N1* | Mauritania | KJ938219 | 100 % |
| *Coptodon zillii voucher Fish-CZ-5* | Pakistan | MF460324 | 100 % |

The phylogenetic analysis for *Coptodon zillii* isolate TZ80 collected from Damietta branch of the River Nile at Dakahlia Governorate using the COX 1 gene is presented in Fig. (4). The phylogenetic tree showed two main clades. The first clade divided into two sub-clades; first sub-clade divided into two sub sub-clades; the first sub-sub-clade includes *Coptodon zillii* isolate TZ80 (Egypt) and *Coptodon zillii* voucher Fish-CZ-10 (Pakistan) with bootstrap support (96% BS), the second sub- sub clade includes *Coptodon zillii* voucher Fish-CZ-4 (Pakistan). The second clade includes *Coptodon zillii* strain N2 (France) and *Coptodon zillii* voucher Fish-CZ-5 (Pakistan) with bootstrap support (99% BS). Fig. (5) illustrates the phylogenetic tree for different analyzed *Coptodon zillii* isolates from Damietta branch of the River Nile, Burullus Lake and Rayyan Lake, Egypt inferred from COX 1 gene sequences obtained from Gene Bank (highlighted in yellow color). Bootstrap tests were performed with 1000 replications.

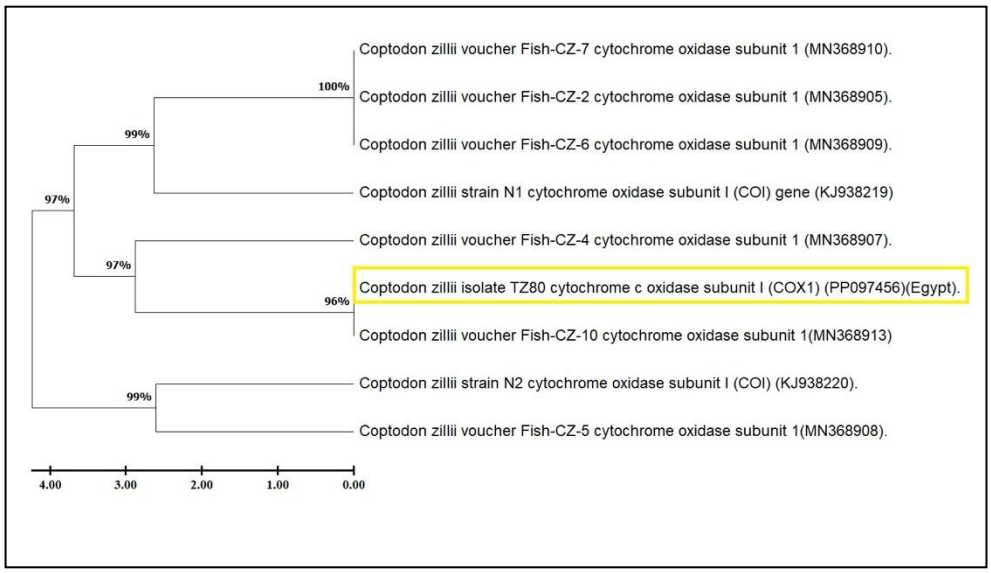


**Fig. 4.** Phylogenetic tree for *Coptodon zillii isolate TZ80* collected from Damietta branch of the River Nile at Dakahlia Governorate – Egypt inferred from COX 1 gene sequences obtained from Gene Bank (highlighted in yellow color). Bootstrap tests were performed with 1000 replications.

## 2. Histopathological Studies on Tilapia zillii (*Coptodon zillii*) infested by Gill Helminthes

**Table 4.** Data generated from Cytochrome C Oxidase Subunit I (COX1) gene for *Coptodon zillii* isolates

| DNA region | Isolates | A | T | G | C | AT % | GC % | GenBank Accession No. | Pairwise Identity (PI) | Nucleotide (bp) |
|---|---|---|---|---|---|---|---|---|---|---|
| Cytochrome C Oxidase Subunit I (COX 1) | *Coptodon zillii isolate TZ77* | 144 | 180 | 131 | 196 | 49.8 % | 50.2 % | PP097389 | 100% | 751 |
| | *Coptodon zillii isolate Cz85* | 138 | 169 | 120 | 192 | 49.6 % | 50.4 % | PP097446 | 100 % | 619 |
| | *Oreochromis niloticus* isolate On7785 | 137 | 169 | 121 | 192 | 49.4 % | 50.6 % | PP085499 | 100% | 619 |

The normal histological features of the gills of redbelly tilapia are shown in Figs. (6-8). The gill filaments are feathery structures, and gill lamella are thin-walled, semi-circular

sheets (Fig. 6). The gill lamellae are arranged as two opposite rows at the superior and inferior sides of the gill filament (Fig. 6). Gill filaments are separated by interfilamental spaces, whereas gill lamellae are separated by interlamellar spaces (Fig. 6). The red coloration penetrating the gill filaments and gill lamellae indicate the branchial blood vessels and their extensions (i.e. blood capillaries) passing across delicate lamellae. As illustrated in Fig. (6), gill lamellae on one side of the gill filament are in opposite direction to corresponding lamellae on the opposite side. As illustrated in a close view of the distal compartment of an individual gill filament (Fig. 7), the gill filament is strengthened by a median cartilaginous plate and holds sophisticated mucous cells in the interlamellar epithelium and sides of the gill lamellae (Fig. 8). Fig. (7) shows the median cartilaginous plate of the gill filament, which comprises chondrocytes in lacunae, surrounded by extra cartilaginous matrix (purple in color) and peripheral, single-layered coat. The cartilage is formed of chondrocytes, matrix (ground substance) and envelope structured by simple squamous epithelium (Fig. 7).

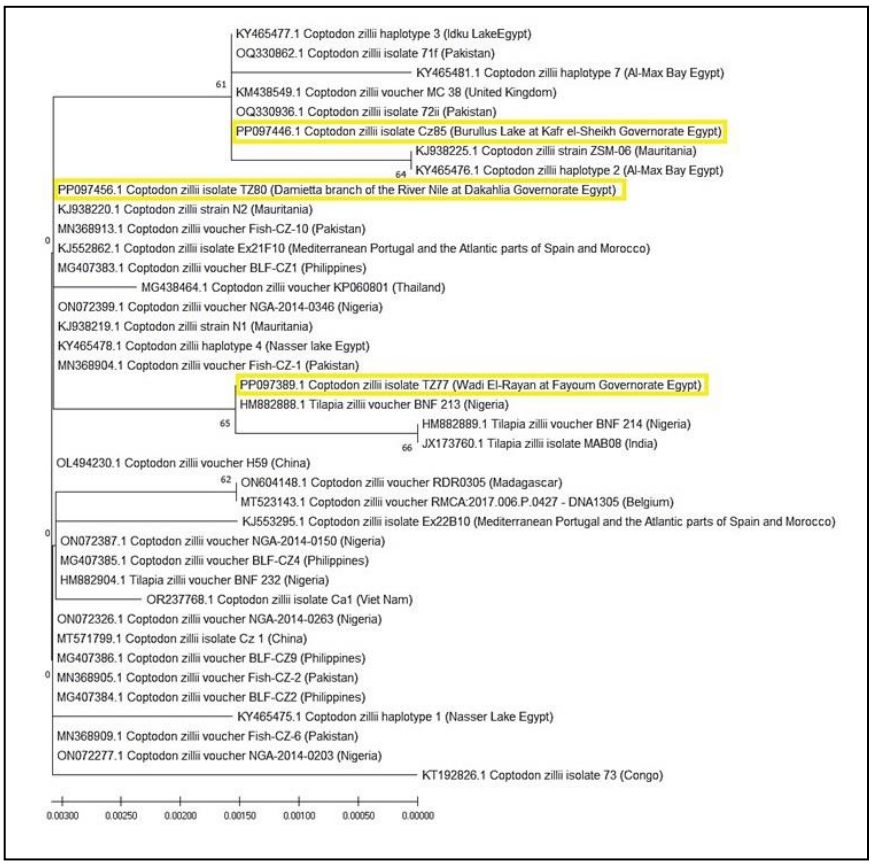


**Fig. 5.** Phylogenetic Tree of *Coptodon zillii* Isolates highlighted in yellow are the identified specimens: TZ77 (Wadi El-Rayan, Fayoum Governorate), Cz85 (Burullus Lake, Kafr El-Sheikh Governorate), and TZ80 (Damietta branch of the River Nile, Dakahlia Governorate). Bootstrap tests were performed with 1000 replications

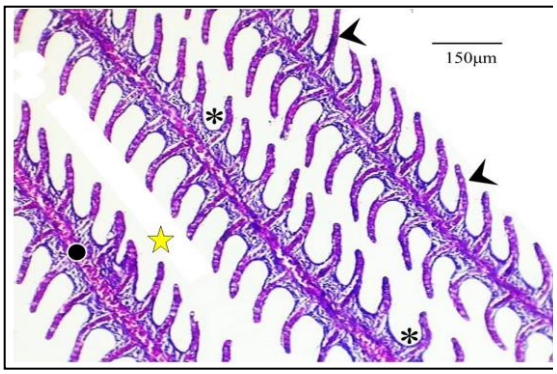


**Fig. 6.** Microscope photograph showing the normal structure of the gill filaments of the redbelly tilapia, *Tilapia zillii* (Gervais, 1848). Note that the gill lamellae (arrowhead) project on both the superior and inferior surfaces of the filament. Gill filaments are separated by interfilamental spaces (yellow Star). Note also that the gill lamellae are separated by arenas known as interlamellar Spaces (asterisk). Scale bar= 150 µm

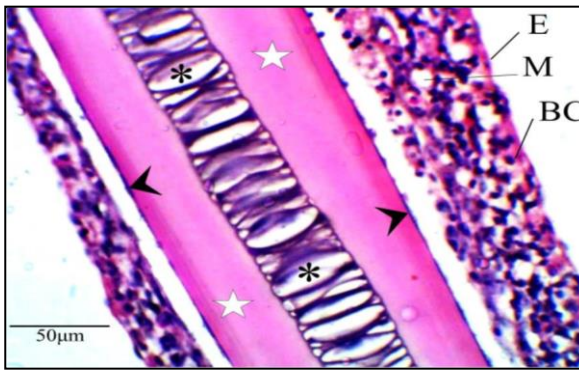


**Fig. 7.** Microscope photograph showing close view of the cartilage supporting the gill filament of *T. zillii*. Note the chondrocytes (asterisk) at the core of the cartilage, which is provided with extracartilaginous matrix (white star) and is enveloped by a simple squamous epithelium (arrowhead). Note the epithelial coat of the filament (E) and the mucus cells (M) which lubricate the filament. Note also that the gill epithelium is enriched by blood capillaries (BC). Scale bar= 50 µm

Three ectoparasites are common on the gills of the red-belly tilapia, namely *Centrocestis formosanus* (Digenea), ancyrocephaline and gyrodactylids (Monogenea) and *Ergasillus sieboldi* (Crustacea: Copepoda). The encysted digenean metacercaria was found to nest inside the cartilage at the gill arch (Fig. 9) or the cartilage penetrating the gill filament. In some infestation patterns, clusters of encapsulated metacercaria were encountered inside the gill arch of the redbelly tilapia. For digenean cysts nesting inside the cartilage crossing the gill filament was observed to create a massive swelling around the encysted metacercaria, mainly structured by proliferating chondrocytes at different stages of development. As illustrated in Fig. (9), the growing metacercaria is obliged to bend its body in order to reside properly inside the space-limited living place. Many local histopathological impacts were recognized at the hot spot of host-parasite interface and comprised deformity of the cartilage, displacement and compression of gill epithelium at the bulging locus, the cartilage is U-turned, and shifted lamellae appeared short and small in size (Fig. 9).

As demonstrated in Figs. (10, 11), the monogenean parasite (*Cichlidoggrus* spp.) inserts its posterior attachment organ (haptor) between two adjacent gill lamellae on the

same gill filament. The adhesive attitude of the monogenean worm is likely up stream, with the body proper bent in a dorsoventral posture, parallel to the longitudinal axis of the gill filament, but perpendicular to the inserted haptor (Figs. 10, 11). The two affected lamellae were seen divergent, with few mucous cells in the vicinity of the haptoral attachment (Fig. 11). The mucous cells are scarce. A fibrous coat is evident around the squeezed metacercaria, as shown in Fig. (12). Clustered digenean larvae caused disaggregation and fragility of the cartilaginous plate inside the gill arch (Fig. 9) .

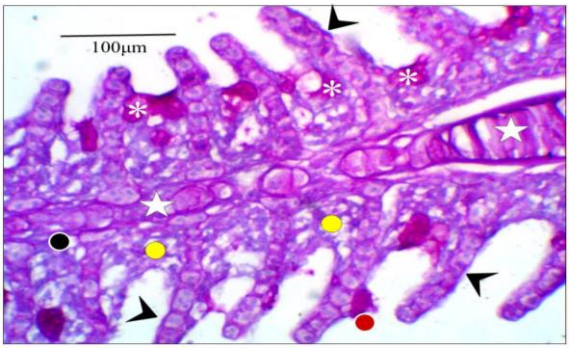


**Fig. 8.** Microscopic photograph showing close view of the gill filament of *Tilapia zillii*. The gill filament holds two rows of gill lamellae (arrowhead) and is strengthened by a median skeletal (cartilaginous) support (white star). Note the distribution of mucus cells in the interlamellar epithelium (asterisk) and lateral surface of gill lamellae (red solid circle). Note also the interlamellar epithelium (yellow solid circle) and undifferentiated cells at the base of the gill epithelium (black solid circle). Scale bar = 100 µm

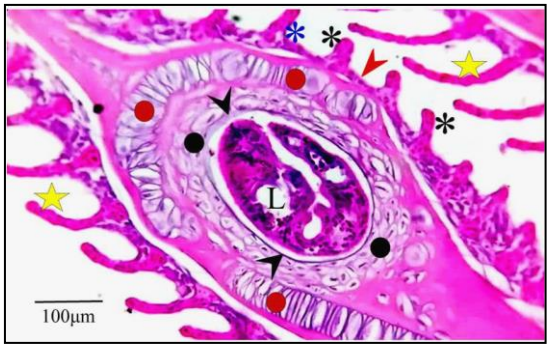


**Fig. 9.** Microscope photograph showing severe damage and tissue modifications created by an individual digenean larva in the gill filament of the redbelly tilapia from Rayyan Lake. Note that the larva (L) is enveloped by a fibrous coat (black arrowhead) and multilayered cartilaginous capsule (black solid circle). Note also marked swelling of the gill area(cartilage and epithelium) invaded by the digenean larva. The gill epithelium is highly compressed (red arrowhead) and the gill lamellae are shortened (asterisk); compare them to gill lamellae on the opposite area of the filament (yellow star). The cartilage supporting the gill filament (red solid circle) is recurved (yellow solid circle) to squeeze the encapsulated larva, leaving the distal area of the gill filament deprived of skeletal support. The mucous cells are scarce (blue asterisk). The gill epithelium in the vicinity of encystment is thinner than gill epithelium distant from proliferating chondrocytes and supporting cells. Scale bar= 100 µm

*Gyrodactylus cichlidarum* was observed to cling to superficial layer(s) of the epithelium utilizing 14 marginal hooklets, in addition to the suction force created by the cup-shaped haptor. A similar haptoral component is owned by the viviparous gyrodactylid

monogenean, *Macrogyrodactylus clarii*. However, the large size and remarkable length of *Macrogyrodactylus* (up to 2.7mm) necessitate the adoption of different nesting tactics. This organism was observed to conceal across the interhemibranchial septum, with the haptor gently and superficially implanted on the hemibranchial area at the proximal sector of the gill filaments.

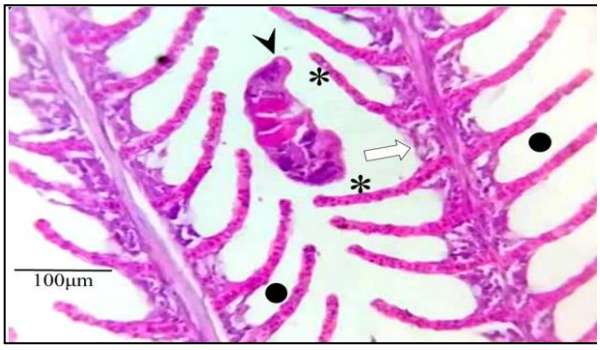


**Fig. 10.** Microscope photograph showing close view of the nesting of a single monogenean worm (arrowhead) between two adjacent gill lamellae of an individual gill filament of the redbelly tilapia. Note the shift in the posture of the two gill lamellae bordering the body of the parasite (asterisk). Note also these lamellae are divergent and widely - spaced than parasite - free lamellae (black solid circle). The mucous cells appear at the infection locus (arrow). Scalebar = 100µm.

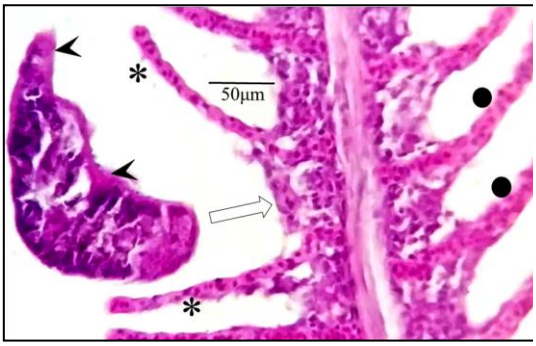


**Fig. 11.** Microscope photograph showing a close view of the nesting episode of an individual monogenean worm (arrowhead) between two adjacent gill lamellae (asterisk) of an individual gill filament of the redbelly tilapia. Note the modification in positioning of the two gill lamellae bordering the body of the monogenean worm (asterisk). Note also that the affected lamellae are widely spaced than parasite-free lamellae (black solid circle). Scale bar = 50µm.

The adhesive attitude and effects of the copepod crustacean, *E. sieboli* on inhabited area of the gill filaments (two or more) are shown in Figs. (12-15). The anterior side of the copepod crustacean is firmly forced against the opercular side of the gill arch with the aid of two 4-segmented structures, namely second antennae.

Worm attached to the proximal (basal) region of the gill filaments generates heavy compression over adjacent gill epithelium and partial to complete lamellar fusion (Figs. 12 and 13). The second antenna was observed to create a deep invagination on the gill filament under effect (Fig. 13). Close to the body of the worm, PAS-positively stained mucous cells were evident (Figs. 12 - 15). Signs of hemorrhage or light bleeding were observed in the vicinity of the host-parasite contact (Fig. 14). Rarely, this ectoparasite was observed to cling to the distal area of the gill filaments (Fig. 15), with the two egg sacs

directed toward the downstream pathway of the gill–ventilating water current. The head region and laterally positioned appendages create pressure and physical displacement of the gill filaments which show signs of degeneration and atrophy (Fig. 15). Adjacent filaments, particularly their distal regions exhibited marked response to the clinging worm in the form of pronounced activity to produce mucous, probably to deter the intruder (Fig. 15). Many active mucous cells were recognized in the deep layers of the epithelium at this hot spot.

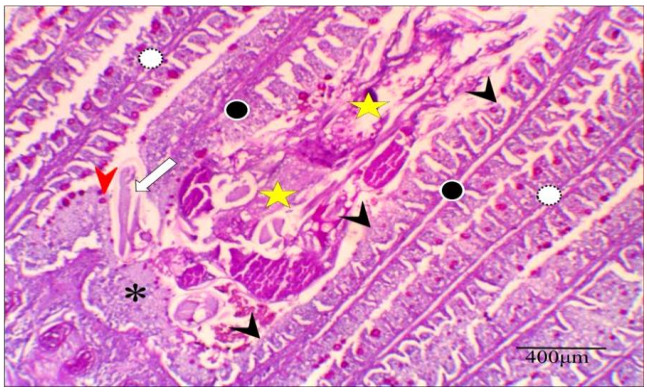


**Fig. 12.** Microscope photograph showing close view of the host (Redbelly Tilapia) – parasite (Copepod) interface. The parasite (yellow star) is nesting between two neighboring gill filaments, with the head region directed towards the gill arch, while the tail region towards the distal parts of the gill filaments. Note the compression of the tissues of the gill filaments in contact with the sides of the parasite, partial to complete lamellar fusion (black arrowhead). Note also that the filaments next to the altered filaments (black solid circle) appear normal (white solid circle). At the site of the effect of the second antennae (arrow), recognize the activity of the mucous cells (red arrowhead). Moreover, the gill epithelium opposite to the anterior most region of the copepod undergoes compression and tissue degeneration (asterisk). Scale bar= 400µm.

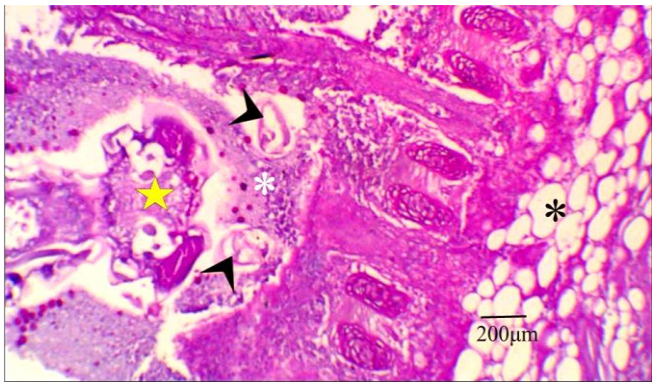


**Fig. 13.** Microscope photograph showing location and orientation of the nesting spot of the gill ectoparasitic copepod on the gills of the Redbelly Tilapia from Rayyan Lake. Note that the anterior head region of the copepod (yellow star) creates compression and erosion of the gill epithelium and partial to complete lamellar fusion (white asterisk). The arrowhead points to a cross section of the second antenna (the principal tool of attachment) and the black asterisk denotes reticular Connective tissue. Scale bar = 200µm.

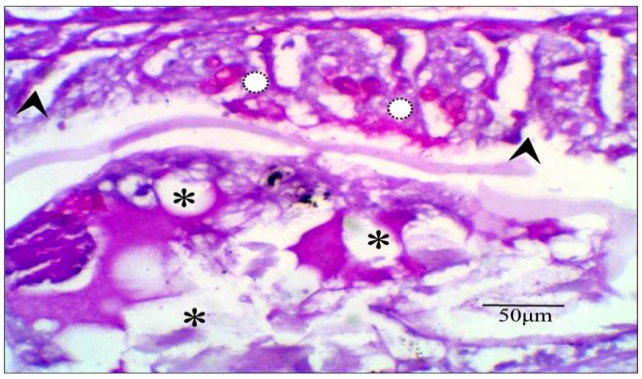


**Fig. 14.** Microscope photograph showing close view of the host-parasite interface (Redbelly Tilapia - copepod system). The body of the parasite (asterisk) compresses and erodes the gill epithelium in contact (arrowhead). Note the aggregation of the mucous cells (white solid circle) at the attachment spot. Note that many lamellae (respiratory units) seem dysfunctioned and lost their original architecture. The red patches of the epithelium likely reflect a hemorrhage due to friction between the copepod and the cichlid fish host. Scale bar= 50µm.

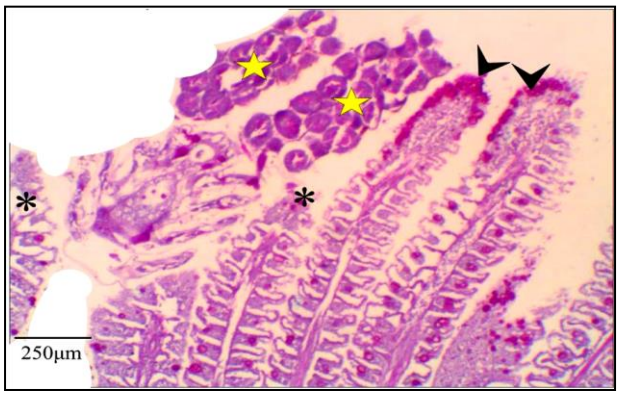


**Fig. 15.** Microscope photograph showing the attachment of the copepod to the distal region of the gill filaments of the redbelly tilapia inhabiting Burullus Lake. Note the marked degeneration, hyperplasia and atrophy of the gill tissues subjected to the tools of attachment of this ectoparasite. (asterisk). The yellow star points to the egg sac of the ectoparasite. Note that the orientation of the worm, with the egg sac at the downstream of the gill ventilating water current in the vicinity of the distal compartment of the gill filaments. Note also the powerful response of the fish host to infestation through the activity of the mucous cells (arrowhead) adjacent to the posterior region of the body of the copepod worm. Scale bar= 250µm.

## DISCUSSION

Cichlid fish, belonging to the family Cichlidae, are among the most varied groups of vertebrates and serve as a prime example in the field of evolutionary biology. They are particularly valuable for studying the mechanisms that facilitate organismal diversification during evolutionary characterization **(Kocher, 2004; Seehausen, 2007, 2015; Turner, 2007; Santos & Salzburger, 2012; Salzburger, 2018)**. A multitude of studies have examined cichlid genomes to address critical questions in evolutionary biology. These questions encompass identification of genomic signatures and potential factors influencing evolutionary characterization, analysis of genetic variation in terms of its quantity and distribution, both within individual genomes and among various species—with the

implications for evolutionary divergence of populations into separate species. Furthermore, researchers aim to understand the processes by which genetic variation is created and maintained, with a particular emphasis on the role of gene flow. As a result, a significant and rapidly expanding collection of genetic and genomic data on cichlids has been compiled. Till 2011, 116 studies have contributed to a total of 51.5 trillion base pairs available in the National Center for Biotechnology Information (NCBI) Sequence Read Archive **(Leinonen *et al*., 2011)**. Nevertheless, creating a comprehensive overview of genomic diversity in cichlids is complicated by differences in methodologies in various studies, which include varying genetic and sequencing techniques, unique evolutionary hypotheses, and targeted investigations into specific cichlid evolutionary characterization. Fish can be ultimate, paratenic, or intermediate hosts throughout the parasite life cycle, despite being the most commonly infected species **(Ali & Ismail, 2022)**.

From the present investigation, each parasite taxon exhibited a pronounced microhabitat specialization on/in the available space resources. While the oviparous monogeneans (*Cichlidogyrus* and *Scutogyrus*) was observed to insert their posterior attachment organ (haptor) between two adjacent gill lamellae on an individual gill filament, cysts of the digenean, *Centrocestis formosanus* that was ascertained to establish a resistant, oval to spherical nest impregnated deeply inside the cartilaginous compartment of the gill arch or gill filaments, while the crustacean copepod, *Ergasillus sieboldi* exerts a strong physical/mechanical pressure against the basal part of the two or more adjacent gill filaments close to the opercular side of the gill arch (i.e. opposite surface of the gill rakers).

Although the haptor of *G. cichlidarum* is equipped with two massive, sharp-pointed hamuli, these structures were rarely involved in deep penetration attitudes into the gill epithelium. It is probable that the cartilage occupying the core of the gill filament acts as a physical barrier hindering the completion of the hamulus task. Instead, this pathogen clings to superficial layer(s) of the epithelium utilizing 14 marginal hooklets radially arranged on the periphery of the haptor, in addition to the suction force created by the cup-shaped haptor. A similar haptoral component is possessed by the viviparous gyrodactylid monogenean, *Macrogyrodactylus clarii*. According to **El-Naggar *et al.* (2001)**, who examined the movement patterns of *M. clarii*, the worm's head region scans the gill filament from its distal region to its proximal region and vice versa. The haptor's attachment to the gill filament's proximal section may allow the worm to travel in this way. However, the large size and profound length of *Macrogyrodactylus* (up to 2.7mm) necessitates the implementation of different nesting tactics. This organism was observed to hide along the interhemibranchial septum, with the haptor gently and superficially implanted on the hemibranchial surface close to the proximal area of the gill filaments.

The pathological changes observed in this study are similar to those documented by **Molnár (1972)** (*Dactylogyrus lamellatus* infecting *Ctenopharyngodon idella*), **Buchmann (2012)** (*Pseudodactylogyrus anguillae* and *Pseudodactylogyrus bini* infecting *Anguilla*

*anguilla*), **Molnár *et al.* (2016)** (*Ancyrocephalus paradoxus* infecting *Sander lucioperca*), **Arya and Singh (2020)** (Mizelleus indicus infecting *Wallago attu*), and **Igeh and Avenant-Oldewage (2020)** (*Cichlidogyrus philander* infecting *Pseudocrenilabrus*), who also reported epithelial cell hyperplasia and subsequent fusion of lamellae, which are a key feature of parasitic infection. These studies also reported hyperplasia of the epithelial cells and the fusion of lamellae that followed. Damaged capillaries were extensively buried in the hyperplastic tissue, thickening the secondary lamellae due to the intense proliferation of interlamellar epithelia.

Likewise to the present investigations, **Abd EL Maged *et al.* (2024)** demonstrated the detrimental impact of monogenean parasites on the gill tissue. The monogenean parasites are invasive and damages host tissue, as evidenced by the severe destruction to the branchial tissue. The anterior haptor's attachment and invasion of the lamellae using its anchor hooks may be the cause of this injury. The fish host reacts to this attachment by either secreting too much mucus or by lamellar hyperplasia with secondary lamellae fusion **(Buchman, 1997)**.

Additionally, the host reacts to monogenean attachment by secreting mucus that is abundant in lectin, antibodies, antimicrobial peptides, and lysosomes **(Nakamura *et al.*, 2000; Smith *et al.*, 2000)**. Attached to parasites, these chemicals aid in immunological detection and draw immune cells such as macrophages and lymphocytes **(Buchmann & Lindenstrøm, 2002)**.

Histopathological studies revealed that the monogenean parasites were distributed throughout interlamellar gaps, accompanied by necrotic lamellar epithelial debris and extensive lamellar epithelium damage. In the gills of *C. gariepinus* infected with *M. clarii* and *P. philander* infected with *C. philander*, there were also excessive mucous cells, which were consistent with **Igeh and Avenant-Oldewage (2020)**, who noted an increase in mucous production with neutrophils. According to **Arafa *et al.* (2009)**, the haptor's attachment resulted in the compression and close packing of nearby gill lamellae, giving the gills an unusual appearance.

This adhesive attitude likely protects the notably large-sized organism from the sweeping effect of the gill-ventilating water currents. The ectoparasites were observed to pick up a number of epithelial cells and sometimes blood cells emerging as a result of the rupture of the blood vessels or capillaries inside the feeding pit created by the physical strain exerted by the mouth opening along with the extracorporeal digestion via proteolytic enzymes secreted by the pharyngeal glands of the worm. A similar feeding habit is likely adopted by the relative species in the Family Gyrodactylidae, namely *G. cichlidarum*. **Molnár (1980)** discovered that *Ancylodiscoides vistulensis* (syn. *T. vistulensis*) attaches between two neighboring lamellae, on one side with the dorsal pair of anchors and on the other side with the ventral pair. This finding is consistent with the present findings. In the

same study, the anchors frequently sank as far as the cartilaginous supporting framework of the gill filament.

The majority of the tissue damage that happens where the anchors pierce the gills, rupturing the epithelial cells and resulting in permanent cellular alterations. The interaction between the parasite's body and the secondary gill lamellae, as well as the penetration of the anchors and marginal hooklets into the gill epithelium, result in pressure and compression causing this pathology. Similar compression has been reported by **Arafa *et al.* (2009)** and **Igeh and Avenant-Oldewage (2020)**.

When a monogenean parasite attaches and feeds on its host, it damages the gills, causing mechanical rupture and gill tissue loss. The ventral and dorsal anchors are primarily used to accomplish the attachment, which normally fixes itself between the gill lamellae. The fixing of marginal hooklets further strengthens the attachment. Many monopisthocotylans, such as *Cichlidogyrus* spp. on *Oreochromis niloticus* **(El-Naggar *et al.*, 2001)**, *Paradactylogyrus* sp. on *Labeo rohita* **(Kaur & Shrivastav, 2014)**, *M. clarii* on *C. gariepinus* **(Arafa *et al.,* 2009)**, and *C. philander* on *P. philander* **(Igeh & Avenant-Oldewage, 2020)** insert their anchors into the interlamellar gill epithelium of their hosts. In certain species, anchors have the ability to pierce and alter the gills' extracellular cartilaginous matrix **(Kaur & Shrivastav, 2014; Igeh & Avenant-Oldewage, 2020)**.

In *A. anguilla* infected with *P. anguillae* and *P. bini*, **Buchmann (2012)** has also noted similar pathogenic host responses, such as hyperplasia of mucous cells and gill epithelial cells and excessive mucus production. The host's defensive mechanisms cause these gill alterations **(Reda & El-Naggar, 2003; Vankara *et al.,* 2022)**. Observations in the present study showed that, although mucus cells are present in the space between the filaments, goblet cells appeared to be noticeably absent. This is in contrast to the majority of authors who have reported an increase in goblet cells **(Reda & El-Naggar, 2003; Molnár *et al.*, 2016)** and mucus cells **(Buchmann, 2012; Arya & Singh, 2020; Igeh & Avenant-Oldewage, 2020; Vankara *et al.*, 2022)**.

Two forms of encystment were encountered for the digenean *C. formosanus*, namely solitary and colonial in the present investigation. The first form of encystment was detected in individual gill filaments (one cyst/filament), particularly at the distal region of the filament. On the other hand, the second form of encystment was found inside the cartilaginous plates of the gill arch. About 10 or more juveniles were observed inside lacunae (one cyst/lacuna) enveloped by a fibrous coat surrounded by cartilaginous capsule (numerous chondrocytes impregnated in matrix). Inside the gill filaments, the cartilaginous capsule manufactured around encysted metacercaria of *C. formosanus* was massive and built up of dozens of chondrocytes of different sizes and varying orders of development. These hot spots firmly pressed on the neighboring epithelium and led to marked compression and modification in the architecture of gill tissues. The gill lamellae

underwent outward shifting and noticeable shrinkage in comparison to normal ones. Under high infestation levels, one might expect collateral swellings in vast numbers of the gill filaments which would be bigger and heavier due to massive load of enlarged cartilaginous capsules around pathogens. Ultimately, the flow pattern of water current over respiratory units will be disrupted, and these units may undergo a dramatic decline in their gas exchange capability, lowering the oxygen saturation order inside the blood and a subsequent interruption of the metabolic pathways occurs.

Numerous investigations have been conducted in many countries, including Sri Lanka, to record lesions in the second intermediate host's gills brought on by *Centrocestus formosanus* metacercariae **(Rezaie *et al.*, 2017)**. According to this study, the gills' chronological lesions were caused by the parasite's invasion and encystment in the gill lamellae, which was followed by the host's reaction to the parasite. likewise noted in the present investigation, the respiratory surface was reduced and the gill architecture was severely distorted as a result of the metacercariae's encystment in the gill filaments and gill arches **(Sumuduni *et al.*, 2018)**.

The crustacean copepod, *E. sieboldi* adopted an amazing adhesive attitude in the present investigation. This gill inhabitant was observed to embrace two closely-spaced filaments at the filament-arch communication zone, with the aid of two long second antennae, each compressing four articulating sclerites terminating with pointed, blade-like segment. The second antenna on one side of the head region of the copepod was found to expand, reaching the gill tissues at the opposite side. Concurrently, the second antenna on the other side of the head region of *E. sieboldi* conducts a similar display. The two antennae overlap, crossing one another to reach the gill tissue on the opposite site and implement an efficient piercing attitude to fix the body of the worm in the most sheltered area of the gill tissues.

The parasite density and the 2nd antenna's penetration of the gill filaments, which results in profound lesions, are responsible for the pathological alterations brought on by copepod infection. Additionally, the gills' epithelial tissue is irritated by swimming legs' setae and spines, and mechanical damage is increased by the parasite's movement along the gill filament through the withdrawal and reinsertion of its antennae. Serious clinical and pathological repercussions are likely to result from infection by the more opportunistic species particularly under stressful circumstances. *Ergasilus* species cause the gills of the infected tilapia to exhibit significant epithelial hyperplasia **(Paperna & Overstreet, 1981)**. Necrosis, on the other hand, happens when the antennae's pincer-like motion narrows blood vessels and stops blood from getting to the filaments' distal portions, resulting in ischaemia, degeneration, and necrosis that gives the appearance of marbling. Additionally, the secondary lamellae atrophy is a result of the pressure that parasites apply attaching to the lateral borders of the gill filaments **(Molnár & Székely, 2004)**.

**Hassan *et al.* (2013)** found that copepod parasite, *Lamproglena monodi* attached to the median part of the gill filaments, near the tips of the filaments. The same observation has been made in other species of the parasite, with *L. monodi* preferring the middle position on the gill arches. The second gill arch on both sides had more *L. hoi* parasites than any other arches **(Austin & Avenant-Oldioaj, 2009)**. However, **Tsotsi *et al.* (2004)** found a higher incidence of *Lamproglena clariae* parasites on the fourth gill on both sides. Some suggestions for why the parasite prefers the middle side of the gill filaments include increased gill surface area and water flow through the gill chamber, which may explain why more parasites are found in these positions. This mode of attachment led to strong compression and degeneration of the embraced gill tissues. In addition, the feeding habit of this copepod as a hematophagous organism (i.e. blood-feeder) can amplify the negative impacts on underlying gill tissues, and even the gill builds up and pattern of blood flow across the respiratory system of the fish host. This observation is in agreement with that of **Bauer *et al.* (1981)**, who noted that research on *Ergasilus* constitutes the primary source of information regarding the alterations caused by parasite copepods on fish gills. For the crucial information on alterations brought on by various *Ergasilu*s species, **Avenant-Oldewage (1994)** provided a detailed description of the destruction and clubbing of the filament tips as well as the proliferation of the gill epithelium at the attachment sites of *Ergasilus*.

Small foci of erosion are produced by ergasilids linked to gill filaments; eating appears to include the secretion of proteolytic enzymes for external digestion. Ergasilid infections in cichlids result in such erosion processes **(Paperna, 1991)**. According to **Kabata (1992)**, irritation frequently causes response hyperplasia of the epithelium, which can spread over wide sections of the gills as the infection worsens. This can lead to lamellae fusion and embedding, which reduces the gills' ability to breathe.

The copepod parasite connected to filaments that showed extensive epithelial hyperplasia is an output similar to what was observed by **Tsotetsi *et al.* (2005)**. In fact, this widespread epithelial hyperplasia increases the amount of epithelial tissue available for copepod feeding. Another way to interpret hyperplasia is via regarding it as the host's attempt to isolate the parasite from the surrounding tissue **(Kabata, 1984)**. However, since it hinders the host's capacity to permit gaseous exchange, the thickening of the epithelial tissue may be detrimental to the organism **(Thatcher, 1998)**. The fish's respiratory issues and decreased viability are caused by this hyperplasia.

A parasite that had previously adhered to certain filaments before moving on to other neighboring filaments may be the cause of the pathological changes that have developed in some filaments. According to **Öktener *et al.* (2008)**, the high infestation with *Lipinia pulchella* and unfavorable environmental conditions in Balıklıgöl, Turkey, could be the cause of fish mortality instances. The hyperplasia of the gill tissue, which would constrict air passageways and impede the host's ability to breathe, could be the cause of the mortality

brought on by *Lamproglena* sp. infestation. Therefore, there is no question regarding the possible harm that this copepod may cause to its host, either directly due to its feeding and attachment habits or indirectly through secondary consequences. Since the gills are engaged in various physiological processes, changes in gill tissue may also have a negative impact on the fish's excretion.

The most noticeable pathological alteration seen in gills infested by *Ergasilus labracis* was epithelial hyperplasia coupled with an increase in mucus cells. It appears that this cellular reaction is nonspecific. In cases where a row or cluster of filaments was impacted, clubbing was also seen at the tips of the filaments of severely diseased gills. Numerous epithelioid cells and variable numbers of tiny mononuclear cells were infiltrated into the substantial epithelial hyperplasia that was the result of clubbing. These histopathological alterations, in which many filaments were merged, are less common and less severe than those reported by **Molnár (1980)**.

Furthermore, widespread lamellar desquamations with partial to complete lamellar fusion were noted, along with indications of haemorrhage or light bleeding around the host-parasite interface. These findings concur with those of **Arafa *et al.* (2009)**, who reported that in addition to gill lamellae fusion, certain lesions at the attachment point result in necrosis and a breakdown of epithelial tissue. Furthermore, **Adawy *et al.* (2016)** discovered hyperplasia of the gill filament epithelium, inflammatory responses, haemorrhage, and death of gill tissues, whereas **Ali and Ismail (2022)** documented erosions and necrosis of the secondary lamellae with extensive mononuclear cell infiltration.

## CONCLUSION

Finally, the study finds that geographical isolation has a major impact on the genetic variety of *Coptodon zillii* (redbelly tilapia) across three Egyptian aquatic systems: Rayyan Lake, Burullus Lake, and the River Nile showing clear evolutionary divergence between sub-populations, with high phylogenetic support (94–100% bootstrap values). The study also emphasizes how gill ectoparasites; monogeneans, digeneans, and copepods show particular microhabitat use, each using different attachment and feeding techniques causing particular histological changes in host gill tissues. These results highlight the importance of environmental heterogeneity in influencing host genetic structure as well as parasite ecological dynamics, hence stressing the interaction between evolutionary adaptation and niche partitioning in aquatic ecosystems. The work offers insightful analysis of the factors influencing host-parasite interactions and biodiversity in fragmented environments.

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