MITOCHONDRIAL DNA VARIATION OF *Tilapia zillii* FROM TWO DIFFERENT SITES IN EGYPT

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ABSTRACT

Tilapia zillii is a fresh water cichlid fish distributed widely in Africa and the Middle East**.** In this study, the genetic variability of natural populations of *T. zillii* inhabiting two different aquatic habitats using mitochondrial DNA sequences had been explored. Meanwhile, chemical and biological analyses of the water collected from the two habitats were performed. The results indicated that the two natural habitats (river nile and Lake Qaron) are isolated chemically and biologically. There was no isolation between the two natural populations of *T. zillii*. Such the absence of isolation did not allow identifying significant genetic differences. Tilapia populations were, therefore,-less affected by the recent severe bottleneck although the lake Qaron had been heavily polluted.

KEYWORDS

Tilapia zillii, population genetics, mitochondrial sequencing, heavy metals contamination, chemical analysis.

INTRODUCTION

Tilapia zillii (Gervais, 1848) is an African and Middle-Eastern native tilapiine fish (Chakrabarty 2004, Szitenberg et al. 2012). *T. zillii* is being Euryhaline, and is able to extend its geographic distribution into habitats of a wide salinity range. *T. zillii's* euryhalinity is thus considered to be a key reason for the wide geographic distribution of the species (Briggs 1985, Stiassny 1991, Szitenberg et al. 2012), in particular in coastal rivers.

T. zillii is a monogamous biparental guarder and substrate brooder with both parents committed to a single nest through a breeding cycle (Bruton and Gophen 1992, Szitenberg et al. 2012).

Heavy metals are sometimes called "trace elements". They are the metallic elements of the periodic table. Heavy metals have become of particular interest in recent decades within the framework of environmental investigation. The contamination of water is directly related to the degree of contamination of our environment. Rainwater collects impurities while passing through the air. Streams and rivers collect impurities from surface run off and through the discharge of sewage, agriculture and industrial effluents; these are carried to the rivers, lakes or reservoirs (Salem et al. 2000). Few studies were done in Faiyum areas; most of them were surrounding lake Qaron to study the effect of heavy metals on biota (El Bahi et al. 2013).

The origin of the recent populations of *T. zillii* is unknown. The present study seeks to understand *T. zillii's* mitochondrial variations in Niklah-Berqash (river nile site) and lake Qaron habitats.

MATERIALS AND METHODS

Taxon sampling

Tilapia zillii (Gervais, 1848) belongs to cichlid family. It is found widely in Africa and the Middle East, but has also been introduced outside its native range. It is an important food fish and sometimes seen in the aquarium trade. Its natural habitats are marginal vegetation and seasonal floodplain streams, lakes, and ponds. It was formerly included in the genus *Tilapia* as *Tilapia zillii*.

T. Zillii measures 2 to 14 cm body length (BL) and has an entirely yellow to grey caudal fin with no dots, developing a greyish caudal fin with dots with increasing size **(Fig. 1)**.

Study sites

Fish were sampled using fishing net from two different sites along the River Nile; Niklah-Berqash, Embaba and Lake Qaron, El-Faiyum.

Embaba is a neighbourhood in northern Giza, Egypt, located west of the Nile within the Giza Governorate. Embaba is also the name of an adjacent administrative center in rural Giza Governorate, which has 19 villages in its jurisdiction.

Niklah-Berqash [30° 10' 12.34" N 31° 03' 07.32" E] is one of the villages belonging to the center of Al-Qanater in Giza Governorate in the Arab Republic of Egypt. According to 2006 statistics, the total population in Niklah was 18,173. It is located near El-Maryotia road. The Nile area is surrounded by a sandy soil with slightly heavy vegetation of short trees and palms **(Fig. 2a)**.
Lake Qaron [29° 29' 02.48" N 30° 39' 16.21" E] is the third largest lake in Egypt and the second most famous one after Lake Nasser in the Southern part of Egypt. It lies some 45 meters below sea level and occupies the lowest, northern section of

the Fayoum depression it is simply referred to as the Berka, which means "the small lake" in Arabic. In reality, Lake Qaron is a huge salty body of water where fresh water can be brought from irrigation system. About 370 million cubic meters of drainage water reach the lake annually The high rate of evaporation has led to a concentration of salts, the lake is now as saline as the seawater. It is surrounded by a heavy vegetation of grass **(Fig. 2b)**.

Water sampling and methodology

Water samples were collected from the two selected sites along the River Nile; Niklah-Berqash, Embaba and Lake Qaron, El-Faiyum.

Chemical analysis of water

Chemical tests were performed to analyze:

- pH and total dissolved salts (TDS) by consort c932.
- Total suspended solids (TSS) by using manual method.
- Chemical oxygen demand (COD) by nanocolor 500 D.
- Carbonate (CO_3^{--}) and bicarbonate (HCO_3^{-}) salts by using titration method.

Biological analysis of water (heavy metals analysis)

Heavy metals analysis was done to analyze aluminum (Al), boron (B), barium (Ba), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), vanadium (V) and zinc (Zn) in mg/L [ppm].

The water samples were analyzed using Inductively Coupled Argon Plasma (ICAP) 6500 Duo, Thermo Scientific at Central laboratory - Water & Soil Analysis Unit, Desert Research Center, El Matariya, Cairo, Egypt. England. 1000 mg/L multi-element certified standard solution, Merck, Germany was used as stock solution for instrument standardization.

DNA extraction, amplification and sequencing

Approximately 100 mg of muscle tissue was cut into small pieces and treated with Qiagen DNA extraction kit following the manufacture protocol. Genomic DNA was electrophoresed in 1% agarose gel to examine the quality of the extracted DNA.

The forward primer: 5`- CGCCTGTTTATCAAAACAT-3` and the reverse primer: 5`- CCGGTCTGAACTCAGATCACG -3` were used for amplification and sequencing. The PCR protocol was set as described by Amer et al. (2013). Sequencing of the PCR products was conducted in Macrogen, Seoul, Korea according to their protocol.

Comparisons with sequences in the GenBank database were achieved in BLASTN searches at the National Center for Biotechnology Information site (http://www.ncbi.nlm.nih.gov).
2006.

ETHICAL CONSIDERATIONS

Our Institutional Animal Care and Use Committee (IACUC) at Zoology Department, Faculty of Science, Cairo University has approved this study protocol from the ethical point of view and according to Animal welfare Act of the Ministry of Agriculture in Egypt that enforces the humane treatment of animals.

RESULTS

Chemical and biological analyses of water

Table (1) and **Fig. 3** showed the chemical analysis of water samples and sample locations. It seemed that the water collected from Lake Qaron was alkaline with pH 8.07 due to the presence of high concentration of bicarbonate salts (HCO_3^-) which was 536.8 mg/L, while the water sample collected from Niklah-Berqash area was neutral (pH= 7.14) with low bicarbonate salts (HCO_3^-) [244 mg/L). No carbonate salts (CO_3^{--}) were detected in both studied areas.

TDS and COD were reported with higher values in Lake Qaron area than in Niklah-Berqash area, which were 1.26 g/L and 372 mg/L respectively.

Higher concentrations of TSS were recorded in Lake Qaron area (444 mg/L), while they were not detected in the other studied area.

The data listed in **Table (2)** and **Fig. 4** contained concentration of heavy metals in mg/L [ppm] and sample locations.

The highest Al and Fe concentrations were reported from Lake Qaron area, which were 1.284 and 1. 247 ppm respectively compared to Niklah-Berqash area.

Ba, Co, Mn, Ni and Zn concentrations were recorded with a slightly increase in Lake Qaron area than in the other area.

The higher concentrations of B, Mo and V were estimated in Lake Qaron area which were < 0.0064, 0.0079 and 0.0218 ppm respectively when compared to Niklah-Berqash area.

The lowest Cd, Cr, Cu and Pb concentrations were detected from both studied areas with similar concentrations, which were < 0.0006, < 0.01, < 0.006 and < 0.008 ppm respectively.

Sequence data analysis

By using Palumbi et al. (1991) conserved universal primers, a partial fragment of 16S rRNA gene in the mitochondrial DNA was amplified and sequenced for *T. zilli* inhabiting two different habitats. Muscle tissues from 4 fish samples from lake Qaron and 3 from the river Nile at the vicinity of Giza governorate were used in this study. Approximately, 546 bp were shown to be identical within populations, however, only one base substitution between the two populations was recorded (A>G). The obtained data was aligned with their tilapias counterparts that are available in the Genbank database. The base composition of the sequenced fragment is as follow: A = 29.2%, C = 26.1%, G = 22.3% and T = 22.4%. Within 545 sequenced bases, A = 159, C=142, G=122 and T= 122 **(Table 3)**.

The pairwise genetic distances among the studied populations are listed in **Table 4**. The distance showed the smallest values within Qaron population (D=0.00183-0.00367) since there is only one base transition (A>G or T>C) among them. Between Qaron and Nile populations, the same genetic distance also was obtained since, as mentioned above, there was only one base transition. Some Qaron samples were identical to the Nile ones indicating that it is difficult to distinguish the two populations to be different. It needs more samples and molecular data to reveal the clear genetic relationship between both of them.

DISCUSSION

Based on the results, the two natural water bodies; Niklah-Berqash (river nile) and Lake Qaron were inferred to be isolated chemically and biologically from each other. Recently, lake Qaron became more contaminated with heavy metals like Al and Fe which were found in higher concentrations. The water was more alkaline with higher values of COD, TDS and TSS than in the other studied habitat. All these factors besides the hypersaline water of lake Qaron affected the size and health of the natural population of tilapia fish.

T. zillii from both habitats could not be differentiated based on molecular data. The present results were in agreement with those results reported by Szitenberg et al. 2012 regarding the samples inhabiting the coastal and the Jordan rivers'.

The lack of isolation between the two natural populations of *T. zillii* inhabiting Niklah-Berqash (river nile) and Lake Qaron respectively could be an artifact of the small captured sample-size of tilapias' fish populations and considered in our analysis, which did not allow us to identify significant differences. In particular, it was highly likely that the *T. zillii* population of Niklah-Berqash (river nile) was larger than that inhabiting lake Qaron and both populations were less affected by recent severe bottleneck although the lake Qaron had been heavily polluted with heavy metals specially Al and Fe, higher COD, TDS and TSS which were have a severe risk on the existence of *T. zillii* populations.

Another explanation for the lack of isolation between both tilapia populations inhabiting Niklah-Berqash (river nile) and Lake Qaron could be the translocation of *T. zillii* from the river nile and cultivated it in ancient natural lake Qaron aquaculture.

CONCLUSION

We concluded that the two natural water bodies; Niklah-Berqash (river nile) and Lake Qaron were inferred to be isolated chemically and biologically from each other.

There was a lack of isolation between the two natural populations of *T. zillii* inhabiting Niklah-Berqash (river nile) and Lake Qaron. Such the absence of isolation did not allow us to identify significant genetic differences. Both tilapia populations were less affected by recent severe bottleneck although the lake Qaron had been heavily polluted which have a severe risk on the existence of *T. zillii* population cultivated in it.

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CONFLICT OF INTEREST

Author stated that there is no conflict of interest.

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EXPLANATIONS OF FIGURES

Fig. 1 *Tliapia zillii*.

Fig. 2 Satellite image of the study sites; Niklah-Berqash, Embaba, Giza governorate, Egypt (a), Lake Qaron, Faiyum governorate (b).

Fig. 3 Histogram of chemical parameters in water samples collected from Niklah-Berqash, Embaba and Lake Qaron, El-Faiyum areas.

Fig. 4 Histogram of heavy metals concentration (mg/L [ppm]) in water samples collected from Niklah-Berqash, Embaba and Lake Qaron, El-Faiyum areas.

Fig. 1. *Tliapia zillii*

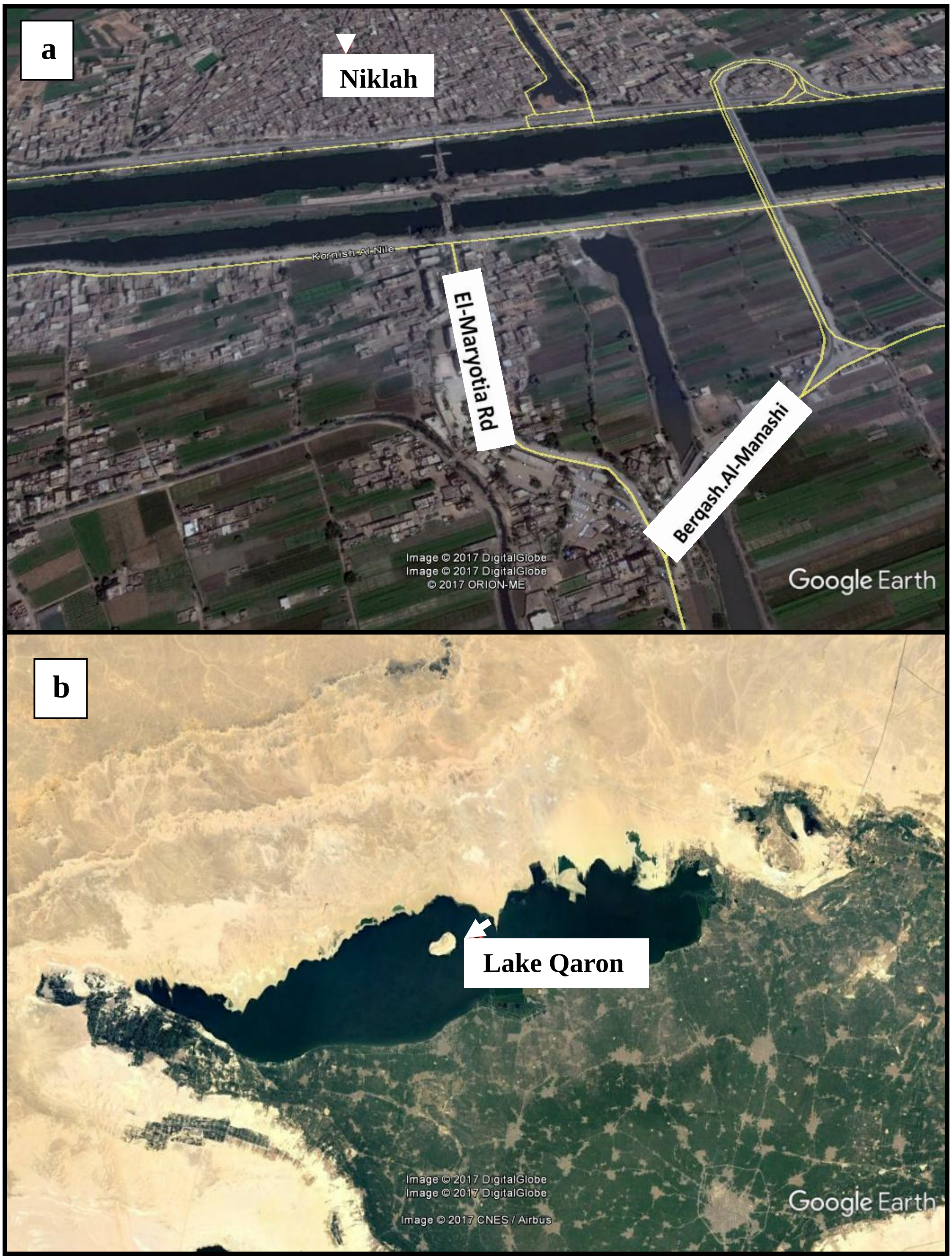

Fig. 2. Satellite image of the study sites; Niklah-Berqash, Embaba, Giza governorate, Egypt (a), Lake Qaron, Faiyum governorate (b).

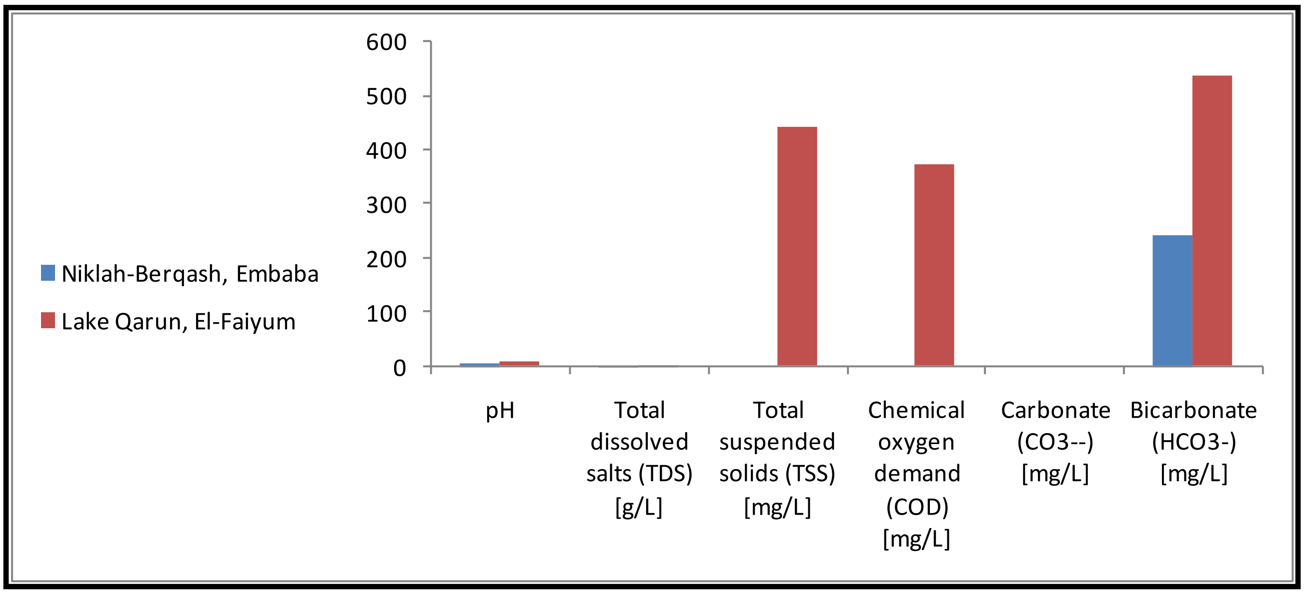

Fig. 3. Histogram of chemical parameters in water samples collected from Niklah-Berqash, Embaba and Lake Qaron, El-Faiyum areas.

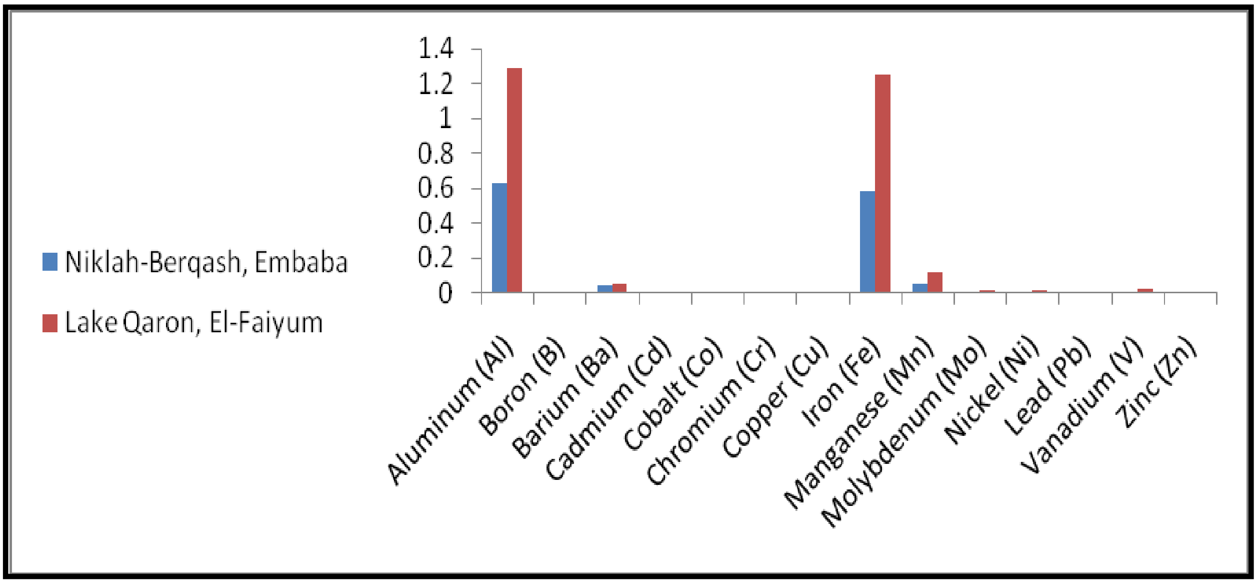

Fig. 4. Histogram of heavy metals concentration (mg/L [ppm]) in water samples collected from Niklah-Berqash, Embaba and Lake Qaron, El-Faiyum areas.

Table (1): Chemical parameters in water samples collected from Niklah-Berqash, Embaba and Lake Qaron, El-Faiyum areas.

Elements	Niklah-Berqash, Embaba	Lake Qaron, El-Faiyum
pH	7.14	8.07
Total dissolved salts (TDS) [g/L]	0.157	1.26
Total suspended solids (TSS) [mg/L]	Nil	444
Chemical oxygen demand (COD) [mg/L]	< 100	372
Carbonate (CO_3^{--}) [mg/L]	Nil	Nil
Bicarbonate (HCO_3^-) [mg/L]	244	536.8

Table (2): Concentration of heavy metals (mg/L [ppm]) in water samples collected from Niklah-Berqash, Embaba and Lake Qaron, El-Faiyum areas.

Elements	Niklah-Berqash, Embaba	Lake Qaron, El-Faiyum
Aluminum (Al)	0.6246	1.284
Boron (B)	< 0.004	< 0.0064
Barium (Ba)	0.0425	0.0532
Cadmium (Cd)	< 0.0006	< 0.0006
Cobalt (Co)	0.0022	0.003
Chromium (Cr)	< 0.01	< 0.01
Copper (Cu)	< 0.006	< 0.006
Iron (Fe)	0.5771	1.247
Manganese (Mn)	0.0472	0.1188
Molybdenum (Mo)	< 0.002	0.0079
Nickel (Ni)	0.0051	0.0082
Lead (Pb)	< 0.008	< 0.008
Vanadium (V)	< 0.01	0.0218
Zinc (Zn)	0.0042	0.0025

Table 3: Molecular diversity of natural populations of *T. zillii* inhabiting river nile and lake Qaron.

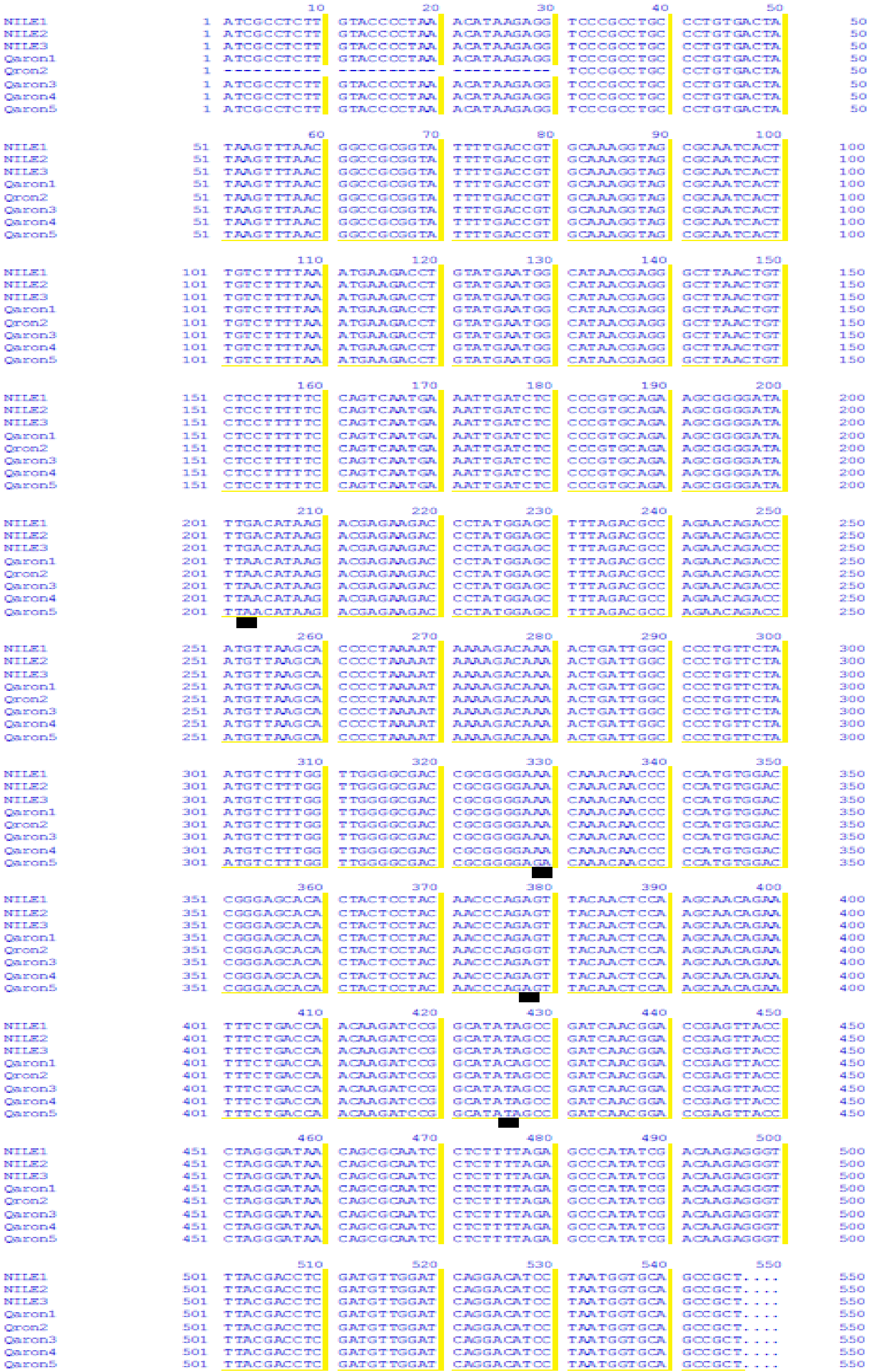

Table 4: Pairwise genetic distances among the different *T. zilli* samples based on the sequenced fragment.

	Nile	Qaron 1	Qaron 2	Qaron 3,4
Nile	--			
Qaron 1	0.037	--		
Qaron 2	0.018	0.038	--	
Qaron 3,4	0.018	0.038	0.018	--
Qaron 5	0.037	0.018	0.018	0.018