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DNA riboprinting analysis of *Tilapia* species and their hybrids using restriction fragment length polymorphisms of the small subunit ribosomal DNA

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Abstract

Morphometric, meristic and DNA riboprinting analyses of *Tilapia* species and their hybrids inhabiting the River Nile were examined. Morphometric data showed striking similarities and overlapping among *Tilapia* species, making it impossible to differentiate these species. Meristic characteristics revealed that *Tilapia* species could be identified into four major groups (*Oreochromis niloticus*, *O. aureus*, *Sarotherodon galilaeus* and *Tilapia zillii*). The lateral line scales differed significantly between the four *Tilapia* species, while the number of fin rays in the dorsal and anal fins differed significantly, differentiating three species (but not between *O. niloticus* and *O. aureus*). Restriction fragment length polymorphisms (RFLPs) of nuclear small sub-unit ribosomal RNA (18S srRNA) gene were used to differentiate the species. Polymerase chain reaction-restriction fragment length polymorphisms data provided a unique pattern for each species with a specific restriction enzyme. Two hybrids of *Tilapia* designated H_1 and H_2 were detected. The endonucleases *Sac*II and *Apa*I differentiated H_1 and H_2. This research revealed a monophylogenetic relationship among all the studied *Tilapia* species.

Keywords: tilapia, 18S srDNA, RFLP, morphometric, meristic, hybrids

Introduction

Tilapia represents the most important group of the family Cichlidae. They constitute a major component of the fish fauna in the River Nile and its tributaries (Rajavarthini, Arunkumar & Michael 2000; Morals, Herrera, Arenal, Cruz, Hernăndez, Pimentel, Guillcn, Martinez & Estrada 2001; Sharaf Eldeen & Abdel-Hamide 2002). They also represent a valuable part of Egypt's national income as food fish. Furthermore, many researchers use *Tilapia* as a fish model for other scientific studies (Abdel-Hamide 1998; Yapi-Gnaore 2001; Sharaf-Eldeen & Abdel-Hamide 2002; El-Serafy, Awwad, Abdel-Hamide & Azab 2003). *Tilapia* are also a successful model for aquaculture (da Silva, Barcellos, Quevedo, de Souza, Kreutz, Ritter, Finco & Bedin 2006). Accordingly, the need to characterize the various species of *Tilapia* are evident. Perdices, Doadrio and Bermingham (2005) considered that the application of molecular techniques would permit enhanced detection of evolutionary structure and taxonomy across the widespread species. They used the mitochondrial DNA to develop an evolutionary history of synbranchid eels. Burridge and Smolenski (2004) also used the sequencing of mitochondrial DNA to distinguish species of families Cheilodactlidae and Latridae and to demonstrate a biogeographical effect.

In the River Nile the reproduction between different *Tilapia* species is feasible and so the production of hybrids could occur. Demarcation among the hybrids is not probable using morphological and meristic characters (Rajavarthini *et al.* 2000). Therefore, this study was conducted to compare classical fish identification and the molecular [restriction fragment length polymorphisms (RFLPs)] method was used to identify the different hybrids of *Tilapia* species.

Materials and methods

Live *Tilapia* fish species (*Oreochromis niloticus*, *O. aureus*, *Sarotherodon galilaeus* and *Tilapia zillii*) were collected from El-Riah El-Tawfequi at Benha City, 50 km north Cairo, between May and August 2000. Morphologically healthy apparent fish measuring

14–16 cm in total length were used for this study (15 for each species).

Morphometric characteristics

Morphometric measurements were computed based on Lagler, Bardach, Miller and Passino (1977) using the following formula:

$$\text{Morphometric index} = \text{Morphometric character}/\text{TL or HL} \times 100$$

Meristic characteristics

The numbers of fin rays were counted in the dorsal fin (DFrs), in the anal fin (AnFrs) and in the caudal fin (CaudFrs). Also, the number of lateral line scales (Lat. Lin. Scales) were counted from the end of the operculum to the end of the caudal peduncle. Fluctuating asymmetry (FA) of the pectoral fin rays, pelvic fin rays and gill rackers (GRs) was carried out by counting the rays or the GRs of the right and left sides. Fluctuating asymmetry was calculated by subtracting the right value from the left one (Sânchez-Galân, Linde, Izquierdo & Garcia-Vazquez 1997).

DNA extraction

Genomic DNA was extracted from liver tissue. The livers of the fish were removed and the liver pieces were stored in the freezer or in 95% ethanol until the DNA extraction started within 1 week. DNA was extracted using the lysis solution. Liver pieces of each species were homogenized and suspended in 500 μL of lysis solution (8 M urea, 2% sodium dodecyl sulphate, 0.15 M NaCl, 0.001 M EDTA, 0.1 M Tris pH 7.5) (Hugo, Stewart, Gast & Byers 1992). Phenol–chloroform extraction was used two to three times to separate the organic and aqueous phases. To precipitate the nucleic acid, iced absolute ethanol was added (2:1 v/v), and left to incubate at $-20\,^{\circ}$C for 24 h. The nucleic acids were recovered by centrifugation at $\sim 5000\ g$ for 15 min. The pellet was dried and suspended in 40 μL of sterile H_2O. One microlitre of the suspended pellet was checked by 0.8% agarose gel electrophoresis for the presence of DNA (Fig. 1).

Determination and amplification of rDNA by polymerase chain reaction (PCR)

A standard PCR mixture was used according to Kessing, Croom, Martin, McIntosh, McMillan and Palumbi (1989). The entire nuclear small subunit ribosomal DNA (srDNA) was amplified using the primers SSU1 (5′-CGACTGGTTGATCC TGCCAGTAG-3′) and SSU2 (3′-TCCTGATCCTTCTAGGTTCAC-5′) (Amresco, Solon, OH, USA) anchored, respectively, in the conserved extremities of the 18S srRNA gene (Stothard & Rollinson 1997). The standard polymerase chain reaction for amplification of nuclear srRNA was carried out 30–35 cycles for 1 min at 94 °C and 3 min at 72 °C.

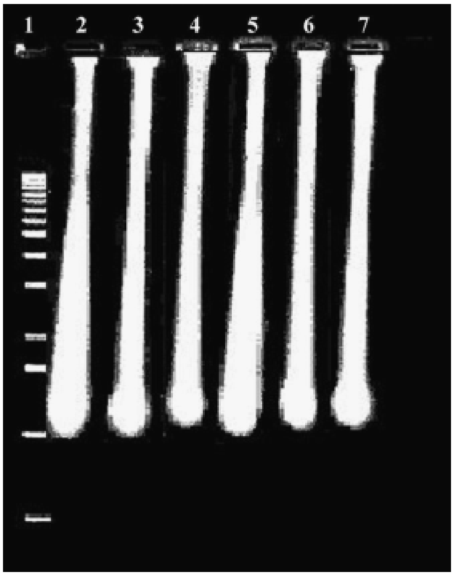

Figure 1 DNA genome from *Tilapia* species. Lane 1 represents 1 kb DNA marker. Lanes 2–7 represent DNA of *T. zillii*, *Oreochromis niloticus*, *O. aureus*, *Sarotherodon galilaeus*, H_1 and H_2 respectively.

Polymerase chain reaction products were isolated after separation by agarose gel electrophoresis [0.8 g agarose; BRL Ultrapure electrophoresis grade/ 100 mL 1 × TAE (Tris base, glacial acetic acid and EDTA)]. Ethidium bromide was used to stain PCR products in the gel (50 μL/100 mL 1 × TAE) for 10 min. The PCR products (bands) were visualized under an ultraviolet (UV) lamp and then cut from the gel. Glass milk DNA purification was used to purify the gene from the agarose gel. Three microlitres of the amplification products were visualized on 0.8% ethidium bromide-stained agarose gels to check the quality of amplification. The remaining 7 μL were mixed with 53 μL of water and divided into 10 μL aliquots for enzyme digestion.

The nuclear 18S srDNA RFLP profiles, in a preliminary test the endonuclease *Bgl*I and *Eco*RI (Amersham Life Science, Kingsville, TX, USA) was evaluated for their ability to differentiate all *Tilapia* species. Additional enzymes were tested including *Sac*II and *Apa*I (Boehringer Mannheim, Baden-Wurtiemberg, Germany) and *Sma*I, *A1w*NI, *Xma*I and *Sst*II (Sigma, St Louis, MO, USA). One microlitre (10–12 U) was used for each digestion reaction, together with 1.2 μL of the relevant enzyme buffer for a final volume of 12.2 μL. The digestion lasted for $\sim$ 3.5 h at $\sim$ 37C, and the digestion products were evaluated on 2% TBE-agarose (FMC Bioproducts, Rockland, ME, USA) gels and stained with ethidium bromide. Band detection was carried out using UV transillumination and then it was photographed (35 mm Kodak film, UK).

Statistical analysis

The data were expressed as mean $\pm$ error and were statistically analysed using Student's *t*-test (Pipkin 1984).

Results

Morphometric characteristic

Sixteen morphometric characteristics of the studied species are tabulated in Table 1. Statistical comparisons of these parameters between every two species are presented in Table 2. *Oreochromis niloticus*, when compared with *O. aureus*, exhibit the lowest significantly differed items among the compared species (five items), i.e. they exhibit similar morphological characteristics. In contrast, *O. aureus* and *T. zillii* recorded the highest significantly differed items (11 items); hence, they are morphologically differed species. *Sarotherodon galilaeus* differed significantly in nine characters when compared with *O. aureus* or *O. niloticus* and in eight characters when compared with *T. zillii*.

The data recorded in Table 2 showed a high similarity coefficient (0.69) when morphometric characteristics between *O. niloticus* and *O. aureus* were compared, whereas, the values of similarity coefficient were < 50% when comparing the rest of *Tilapia* species. This indicates that *O. niloticus* and *O. aureus* are closely similar in their morphological characters.

Meristic characteristics

Seven meristic characteristics were selected in this study. The data are tabulated in Tables 3–5. The number of fin rays in the DFrs and AnFrs differed significantly when all species were compared, aside from *O. aureus* and *O. niloticus*. This is an indication of the same origin for these species.

The number of scales in the Lat. Lin. scales differed significantly between all studied *Tilapia* species; hence, it could be used for *Tilapia* species differentiation. The remaining meristic items were not signifi-

Table 1 Morphometric indices (mean $\pm$ SE) of different *Tilapia* species

	Oreochromis niloticus		***O. aureus***		***Sarotherodon galilaeus***		***Tilapia zillii***	
Morphometric ratio	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
1 – in total length								
SL/TL	81.55	± 0.25	81.45	± 0.26	80.32	± 0.37	82.41	± 0.41
HL/TL	25.53	± .32	25.80	± 0.20	25.08	± 0.16	23.94	± 0.30
BD/TL	36.04	± 0.48	35.15	± 0.37	37.59	± 0.30	33.23	± 0.40
PrDFL/TL	27.09	± 0.50	27.24	± 0.48	26.53	± 0.21	28.03	± 0.34
PrPectFL/TL	29.26	± 0.30	30.31	± 0.23	28.22	± 0.37	27.89	± 0.29
PrPelvFL/TL	34.11	± 0.50	34.29	± 0.36	32.49	± 0.30	32.33	± 0.42
PrAnFL/TL	59.64	± 0.20	58.68	± 0.23	57.82	± 0.43	58.02	± 0.36
LDF/TL	51.88	± 1.22	48.87	± 0.22	48.47	± 0.73	48.80	± 1.30
LpectF/TL	26. 43	± 0.51	27.0	± 0.32	26.78	± 0.88	21.38	± 0.42
LpelvF/TL	18.31	± 0.53	19.40	± 0.13	18.33	± 0.41	17.64	± 0.38
LAnF/TL	17.76	± 1.16	14.51	± 0.20	16.09	± 0.58	18.74	± 1.23
PedL/TL	10.52	± 0.24	10.94	± 0.28	10.34	± 0.25	13.88	± 0.12
PedD/TL	13.02	± 0.22	12.81	± 0.17	14.54	± 0.16	12.45	± 0.35
2 – in head length								
PrOL/HL	31.24	± 0.51	30.78	± 0.68	31.50	± 0.52	35.24	± 0.66
ED/HL	29.54	± 0.44	27.58	± 0.37	29.16	± 0.38	30.10	± 0.45
HD/HL	111.49	± 2.59	110.29	± 1.46	121.35	± 1.30	116.16	± 1.53

Table 2 Significance (*t*-test) among different morphometric indices of different *Tilapia* species

Morphometric ratio	O.n. × O.au.	O.n. × S.g.	O.n. × T.z.	O.au. × S.g.	O.au. × T.z.	S.g. × T.z.
1 – In total length						
SL/TL	0.2794	2.7437*	1.8048	2.5019*	2.0031	3.7995*
HL/TL	0.7221	1.2736	3.6580*	2.7986*	5.1534*	3.3568*
BD/TL	1.4791	2.7531*	4.5478*	5.1147*	3.5507*	8.7671*
PrDFL/TL	0.2179	1.0361	1.5608	1.3677	1.3546	3.7719*
PrPectFl/TL	2.7290*	2.1545*	3.2752*	4.7284*	6.5299*	0.6999
PrPelvFL/TL	0.2926	2.7840*	2.7442*	3.7916*	3.5345*	0.3092
PrAnFL/TL	3.1418*	3.8622*	3.8937*	1.7767	1.5330	0.3569
LDF/TL	2.4366*	2.4057*	1.7310	0.5264	0.0532	0.2216
LPectF/TL	0.9493	0.3453	7.6263*	0.2365	10.9607*	5.5600*
LPelvF/TL	1.9802	0.0296	1.0174	2.4677*	4.3282*	1.2223
LAnF/TL	2.7580*	1.2844	0.5782	2.5665*	3.3849*	1.9411
PedL/TL	1.1526	0.5186	12.7062*	1.6015	9.8094*	12.7139*
PedD/TL	0.9966	7.4327*	1.5307	7.3434*	0.9223	5.4005*
2 – in head length						
PrOL/HL	0.5375	0.8399	0.3569	4.7895*	4.6968*	4.4696*
ED/HL	3.4189*	2.9841*	0.6589	0.8915	4.3066*	1.5974
HD/HL	0.4042	5.6582*	3.4033*	1.5547	2.7829*	2.5857
Similarity coefficient	0.69	0.44	0.5	0.44	0.31	0.44

Number of tested fish = 15.
*Significant at $P<0.05$.
O.n., *Orechromis niloticus*; *O.au.*, *Oreochromis aureus*; *S.g.*, *Sarotherodon galilaeus*; *T.z.*, *Tilapia zillii*.

Table 3 Meristic characteristics (mean ± SE) of different *Tilapia* species

	Orechromis niloticus		O. aureus		Sarotherodon galilaeus		Tilapia zillii	
Meristic Count	Mean	SE	Mean	SE	Mean	SE	Mean	SE
DFrs	29.57	± 0.13	29.65	± 0.12	28.95	± 0.17	27.0	± 0.08
AnFrs	12.07	± 0.12	12.35	± 015	14.05	± 0.20	11.35	± 0.15
CaudFrs	16.79	± 0.11	16.25	± 0.11	16.05	± 0.10	16.30	± 0.14
Lat. Lin. Scales	33.11	± 0.15	33.65	± 0.20	32.24	± 0.15	31.0	± 0.27

DFrs, dorsal fin; AnFrs, anal fin; CaudFrs, caudal fin; Lat. Lin. Scales, lateral line scales.

Table 4 Significance (*t*-test) among different meristic characteristics of different *Tilapia* species

Meristic Count	O.n. × O.au.	O.n. × S.g.	O.n. × T.z.	O.au. × S.g.	O.au. × T.z.	S.g. × T.z.
DFrs	0.4516	2.9443*	17.1664*	3.3552*	17.9618*	10.4873*
AnFrs	1.4805	8.3885*	3.8070*	6.7586*	4.8045*	10.7343*
CaudFrs	3.5200*	5.1269*	2.7343*	1.3498	0.2743	1.4307
Lat. Lin. Scales	2.1468*	3.5769*	6.8399*	5.0123*	7.8108*	3.7218*
Similarity coefficient	0.5	0.0	0.0	0.25	0.25	0.25

*Significant at $P<0.05$.
O.n., *Orechromis niloticus*; *O. au.*, *Oreochromis aureus*; *S.g.*, *Sarotherodon galilaeus*; *T.z.*, *Tilapia zillii*; DFrs, dorsal fin; AnFrs, anal fin; CaudFrs, caudal fin; Lat. Lin. Scales, lateral line scales.

cantly different between the matching compared species.

The degree of similarity between *O. niloticus* and *O. aureus* reached to 0.5 in its meristic characters indicating that these species are closely related (Table 4). Zero similarity coefficients were recorded when comparing *O. niloticus* with *S. galilaeus* and *O. niloticus* with *T. zillii*. Also, very low similarity coefficients

Table 5 Fluctuating asymmetry (FA) of different *Tilapia* species

	Pectoral fin rays				Pelvic fin rays				Gill rackers			
Species	**Right**	**Left**	**FA**	**%FA**	**Right**	**Left**	**FA**	**%FA**	**Right**	**Left**	**FA**	**%FA**
O.n.	13.36	13.50	0.14	13.3	6.0	6.0	0.0	0.0	30.50	30.21	1.29	60
Mean ± SE	0.19	0.13	0.09		0.0	0.0	0.0		0.45	0.34	0.32	
O. au.	13.7	13.7	0.10	13.3	6.0	6.0	0.0	0.0	32.75	32.05	1.20	100
Mean ± SE	0.12	0.12	0.08		0.0	0.0	0.0		0.46	0.53	0.19	
S. g.	12.90	12.90	0.0	0.0	6.0	6.0	0.0	0.0	26.80	25.95	1.43	100
Mean ± SE	0.11	0.11	0.0		0.0	0.0	0.0		0.47	0.51	0.29	
T. z.	13.45	13.45	0.10	6.0	0.0	0.0	0.0	14.95	14.95	15.0	0.40	53.3
Mean ± SE	0.13	0.15	0.08	0.0	0.0	0.0		0.35	0.35	0.30	0.17	

Number of tested fish = 15.
O.n., *Orechromis niloticus*; *O. au.*, *Oreochromis aureus*; *S.g.*, *Sarotherodon galilaeus*; *T.z.*, *Tilapia zillii*.

were reported when comparing the meristic characteristics of *O. aureus* with *S. galilaeus*, *O. aureus* with *T. zillii* and *S. galilaeus* with *T. zillii*, indicating that these three species display great degree of differences.

Fluctuating asymmetry of the pectoral, pelvic fins and the GRs is presented in Table 5. The data showed a fluctuation between the right and the left sides in the number of pectoral fin rays (PectFrs) in all *Tilapia* species except *S. galilaeus*. The pelvic fin rays (PelvFrs) of all *Tilapia* species are bilaterally identical (FA = 0). In contrast, the FA of the GRs are highly represented in all *Tilapia* species.

RFLP of 18S srRNA gene

The PCR-RFLP technique was used to identify the various *Tilapia* species in the River Nile (El-Serafy *et al.* 2003). The DNA genome of *Tilapia* species and their hybrids are represented in Fig. 1. The PCR products of 18S srRNA gene for *Tilapia* species and their hybrids appeared at a length ~ 2000 bp (Fig. 2).

*Eco*RI and *Bg*lI restriction endonucleases did not differentiate between the different strains of *Tilapia* species (Figs 3 and 4). *Eco*RI restriction enzyme grouped the species in one cluster when it fragmented their rRNA gene into two cuts (~ 1650 and ~ 350 bp; Fig. 3). The genes of all species were fragmented into two fragments (~ 1250 and ~ 750 bp; Fig. 4) when digested with *Bg*lI restriction endonuclease. *Bg*l1, *Eco*RI, *Sac*II, *Apa*I, *Sma*I, *A1w*NI, *Xma*I and *Sst*II endonucleases differentiated the *Tilapia* species and their hybrids uniquely (Figs 1–10). The restriction enzyme, *Sma*I, cut the studied gene of *T. zillii* into two fragments (~ 1250 and ~ 950 bp), while the other *Tilapia* species genes were not fragmented (Fig. 5).

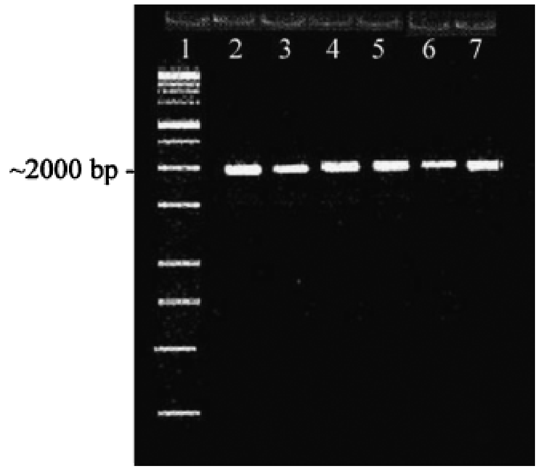

Figure 2 Polymerase chain reaction-restriction fragment length polymorphisms patterns of 18SsrRNA gene of *Tilapia* species. Lane 1 represents 1 kb DNA marker. Lanes 2–7 represent the gene pattern of *T zillii*, *Oreochromis niloticus*, *O. aureus*, *Sarotherodon galilaeus*, H_1 and H_2 respectively.

The 18S srRNA gene of *O. niloticus* was digested into two distinct bands (~ 1750 and ~ 300 bp) by using the enzyme *A1w*NI (Fig. 6), whereas the gene was not split in the other *Tilapia* species. Only the *O. aureus* 18S srRNA gene was digested by the enzyme *Xma*I producing two fragments (~ 1100 and ~ 900 bp; Fig. 7). *Sst*II restriction endonuclease digested the 18S DNA of *S. galilaeus* into two fragments (~ 1600 and ~ 400 bp), whereas the same restriction enzyme fragmented the genes of the rest of the species into three restriction bands (~ 1050, ~ 600 and ~ 350 bp) (Fig. 8).

The same restriction enzyme digested the gene of the other species into three restriction fragments

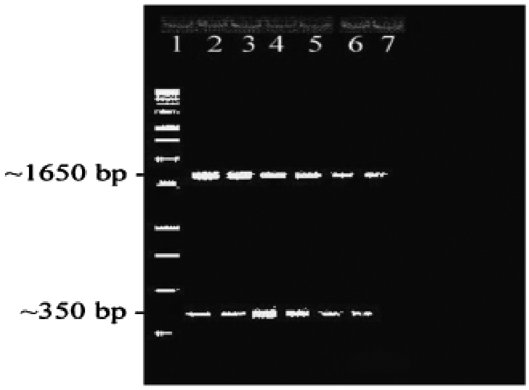

Figure 3 Polymerase chain reaction-restriction fragment length polymorphisms patterns of 18SsrRNA gene restricted by enzyme *Eco*RI. Lane 1 represents 1 kb DNA ladder. Lanes 2–7 represent the gene pattern of *Tilapia zillii*, *Oreochromis niloticus*, *O. aureus*, *Sarotherodon galilaeus*, H_1 and H_2 respectively.

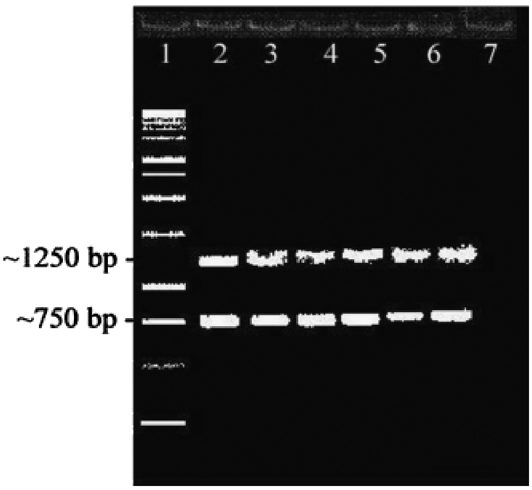

Figure 4 Polymerase chain reaction-restriction fragment length polymorphisms patterns of 18SsrRNA gene restricted by enzyme *Bgl*I. Lane 1 represents 1 kb DNA ladder. Lanes 2–7 represent the gene pattern of *Tilapia zillii*, *Oreochromis niloticus*, *O. aureus*, *Sarotherodon galilaeus*, H_1 and H_2 respectively.

(~ 1000, ~ 650 and ~ 350 bp; Fig. 9). The *Sac*II enzyme cuts the examined gene of H_1 into two fragments, which appeared at ~ 1650 and ~ 350 bp. Only two bands were obtained for the species H_2 (~ 1200 and ~ 800 bp). The same enzyme cut the gene of *T. zillii*, *O. niloticus*, *O. aureus*, *S. galilaeus* and H_1 into three bands with lengths ~ 950, ~ 800 and ~ 250 bp (Fig. 10). Using the enzyme *Apa*I, H_2 can be

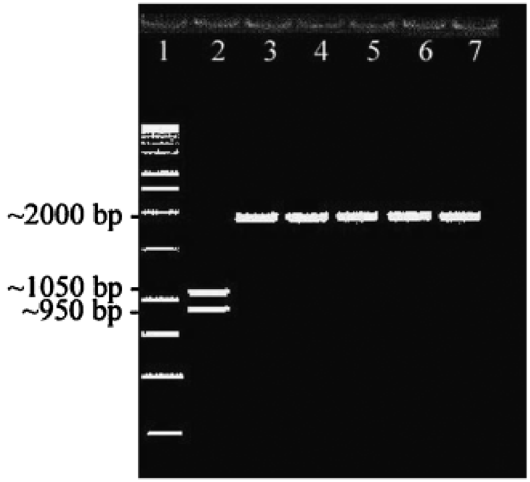

Figure 5 Polymerase chain reaction-restriction fragment length polymorphisms patterns of 18SsrRNA gene restricted by enzyme *Sma*I. Lane 1 represents 1 kb DNA ladder. Lanes 2–7 represent the gene pattern of *Tilapia zillii*, *Oreochromis niloticus*, *O. aureus*, *Sarotherodon galilaeus*, H_1 and H_2 respectively.

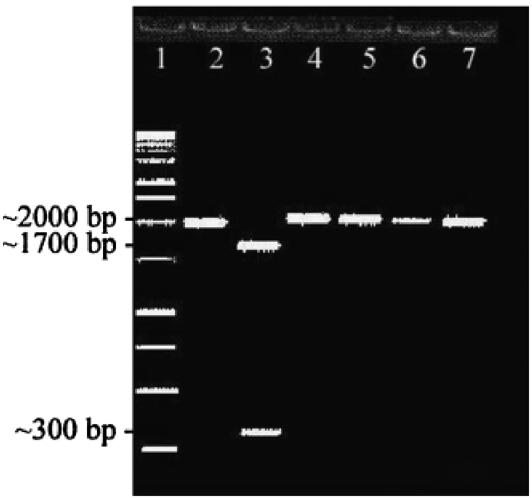

Figure 6 Polymerase chain reaction-restriction fragment length polymorphisms patterns of 18SsrRNA gene restricted by enzyme *Alw*NI. Lane 1 represents 1 kb DNA ladder. Lanes 2–7 represent the gene pattern of *Tilapia zillii*, *Oreochromis niloticus*, *O. aureus*, *Sarotherodon galilaeus*, H_1 and H_2 respectively.

separated from *Tilapia* species inhabiting the River Nile.

Discussion

The identification of fish species including *Tilapia* depends on morphometric and meristic characteristics

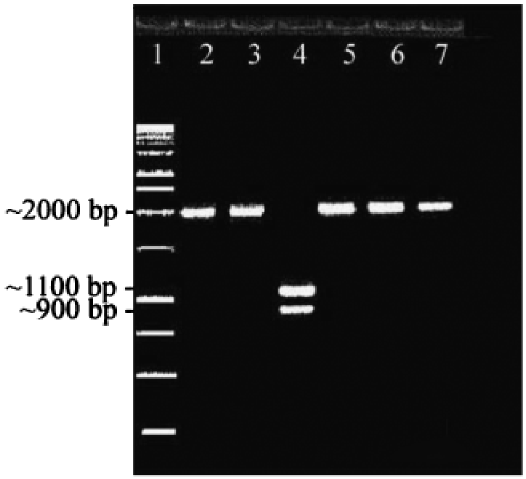

Figure 7 Polymerase chain reaction-restriction fragment length polymorphisms patterns of 18SsrRNA gene restricted by enzyme *Xma*I. Lane 1 represents 1 kb DNA ladder. Lanes 2–7 represent the gene pattern of *Tilapia zillii*, *Oreochromis niloticus*, *O. aureus*, *Sarotherodon galilaeus*, H_1 and H_2 respectively.

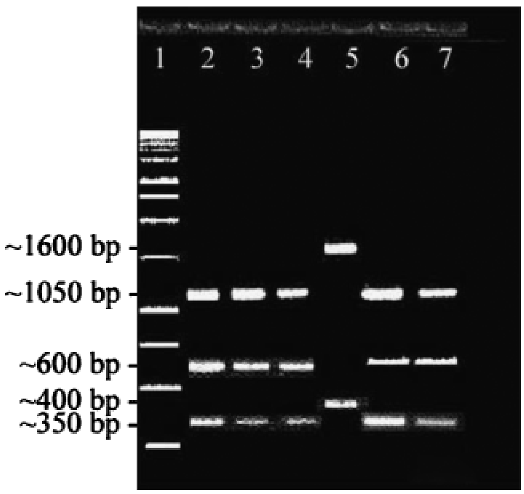

Figure 8 Polymerase chain reaction-restriction fragment length polymorphisms patterns of 18SsrRNA gene restricted by enzyme *Sst* II. Lane 1 represents 1 kb DNA ladder. Lanes 2–7 represent the gene pattern of *Tilapia zillii*, *Oreochromis niloticus*, *O. aureus*, *Sarotherodon galilaeus*, H_1 and H_2 respectively.

of body parts (Yapi-Gnaore 2001). The morphological identification of *Tilapia* species is complicated by the extensive intraspecific variation measurements used for quick identification (Alberston, Market, Danley & Kocher 1999). The results of the present work indicated a great morphological identity among the three

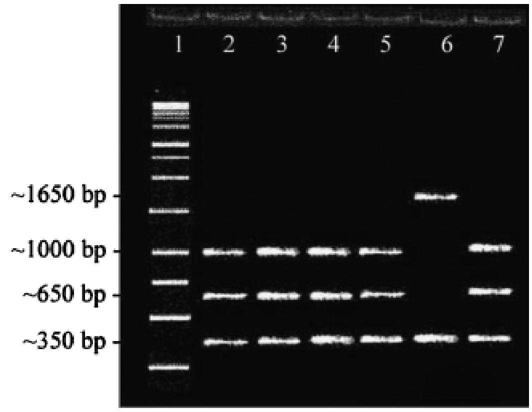

Figure 9 Polymerase chain reaction-restriction fragment length polymorphisms patterns of 18SsrRNA gene restricted by enzyme *Sac* II. Lane 1 represents 1 kb DNA ladder. Lanes 2–7 represent the gene pattern of *Tilapia zillii*, *Oreochromis niloticus*, *O. aureus*, *Sarotherodon galilaeus*, H_1 and H_2 respectively.

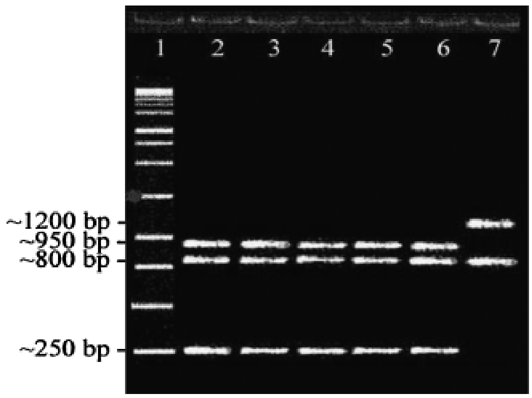

Figure 10 Polymerase chain reaction-restriction fragment length polymorphisms patterns of 18SsrRNA gene restricted by enzyme *Apa*I. Lane 1 represents 1 kb DNA ladder. Lanes 2–7 represent the gene pattern of *Tilapia zillii*, *Oreochromis niloticus*, *O. aureus*, *Sarotherodon galilaeus*, H_1 and H_2 respectively.

Tilapia genera *Oreochromis*, *Sarotherodon* and *Tilapia*. Regarding the data of morphometric and meristic characteristics, two species emerge as closely related; e.g., *O. niloticus* and *O. aureus*, suggesting that they could be monophyletic species, i.e., derived from the same genus (El-Serafy *et al.* 2003). The same phenomenon was previously reported by Oberst, Abban and Villwock (1996); they differentiated three species of genus *Tilapia* (*T. dageti*, *T. zillii* and *T. guineensis*).

The systematic distance between the species is the main factor for the reproductive behaviour barrier

(Barman, Barat, Yadav, Banerjee, Meher, Reddy & Jana 2003). In this study, the monophylogenetic relationship between the genus *Oreochromis* and the genus *Sarotherodon* was recorded. Both are mouth-breeding species. For this reason, natural hybridization between them is possible with a concomitant propagation of *Tilapia* hybrids in the River Nile habitat (El-Serafy, Al-Zahaby, Rizkalla, Labib & Badawy 1994). The analysis of morphometric and meristic characteristics can differentiate among species, but not strains or hybrids (Schönhuth, A'lvarez, Rico, Gonza'lez, Gouveia, Lorenzo & Bautista 2005). The results indicated a lower degree of similarity between genus *Tilapia* and the other two genera, showing a polyphyletic species. This phenomenon was recorded previously in the case of synbranchid eel genera in different habitat (Perdices *et al.* 2005).

Furthermore, the data of FA of the GRs separate *Tilapia* species into three groups that present confusion between *O. niloticus* and *O. aureus* and a higher degree of similarity between *O. aureus* and *S. galilaeus*. The observed data probably distinguished *T. zillii* as a separate group with less degree of similarity. According to FA, *Tilapia* species can be sorted into three groups. This result coincides with the results of Falk, Abban, Oberst, Villwock, Pullin and Rewrantz (1996) and Oberst *et al.* (1996).

Rognon and Guyomard (2003) stated that the morphological parameters of fish are influenced by both genetic and environmental factors. For this reason, molecular techniques based on PCR-RFLP analysis of the 18S srRNA gene have been extensively used as a precise tool of species identification of fish (Fernandez, Garcia, Asensio, Bodreguez, Gonzalez, Hernandez & Martin 2001; El-Serafy *et al.* 2003; Perdices *et al.* 2005). Farias, Orti, Sampaio, Schneider and Meyer (1999) and El-Serafy *et al.* (2003) used RFLP-PCR products of nuclear and mitochondrial DNA as a tool to identify *Tilapia* species. According to the previous authors, some restriction endonucleases (*Eco*RI and *Bgl*I) could not differentiate the studied *Tilapia* species. This suggests that *Tilapia* species could be monophylogenic. Nevertheless, the results of El-Serafy *et al.* (2003) indicated that the restriction enzyme *Sma*I differentiated all species as one group except for *T. zillii*. This confirmed the monophylogenetic relationships of all species except *T. zillii*, which displays a polyphylogenetic relationship. In this study, by using the endonuclease *Sac*II the RFLP profile distinguishes H_1 from the rest of the examined species; hence, this enzyme is specific for H_1 gene. It could be possibly used for H_1 identification. On the other hand, the data obtained after using the endonuclease *Apa*I are characteristic for the H_2 gene. The RFLP data distinguished H_2 from the other *Tilapia* species, so it could be a useful tool in identifying H_2 fish species. Sequencing PCR fragments has become a standard technique in laboratories applying recombinant DNA technologies. Several authors declared that the RFLP option is simpler and faster in addition to its minimal cost (Ram, Ram & Baidoum 1996; Cespedes, Garcia, Carrera, Gonzalez, Sanz, Hernandez & Martin 1998; Quinteiro, Sotelo, Rehbein, Pryde, Medina, PerezMartin, ReyMendez & Mackie 1998).

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