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DNA Barcoding of Israeli Indigenous and Introduced Cichlids

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Abstract

The objectives of this study were barcoding and taxonomic analysis of the five tilapiine species (*Oreochromis aureus, O. niloticus, O. mossambicus, Sarotherodon galilaeus*, and *Tilapia zillii*), two tilapia hybrid strains (Florida red tilapia and Philippine red tilapia), and two endemic wild cichlids (*Tristramella simonis* and *Astatotilapia flaviijosephi*) available in Israel, as well as *O. urolepis hornorum*. Cytochrome oxidase subunit I (COI) 619 bp sequence traces of 104 individuals were assembled, aligned, and compared (GenBank project GI 209553463). The DNA sequences of two hybrid strains were identical to those of *O. hornorum* and *O. aureus*. Absence of intra-specific variability was detected in the commercially used species, *O. aureus, S. galilaeus*, *O. mossambicus*, and *O. urolepis hornorum*. Two DNA sequence variants were detected in *O. niloticus* originating from Ghana and Egypt. In contrast, 2-3 variants were detected in the DNA of each of the non-commercial species. Amino-acid sequences were identical in all "true tilapias" and different from the sequences in the endemic cichlids. As a whole, the protein phylogenetic tree fitted the expected conventional taxonomy as opposed to the respective DNA-based tree. Sequences FJ348047-FJ348150 were submitted to GenBank via the BOLD database (identical to FISH001-08 - FISH104-08 in this database).

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Introduction

DNA sequence analysis has been used for over 30 years to assist in species identification, but different sequences have been used for different taxonomic groups (Ward et al., 2005). The conserved sequence of the 5' region of the mitochondrial gene cytochrome oxidase subunit I (COI or cox1) was proposed as a platform for the universal DNA barcoding of life (Hebert et al., 2003). Of the estimated 670,000 extant species in the world, more than 49,000 have already been formally characterized with DNA barcodes and are registered in the Barcode of Life Data System (BOLD, www.barcodinglife.org). Under that system, the Fish Barcode of Life initiative (FISH-BOL, www.fishbol.org) focuses on fish species, comprising half the global vertebrate species. Currently, barcodes of more than 5,600 out of 30,000 fish species are included in the database.

Tilapiine species (family Cichlidae) inhabit the fresh and brackish water of Africa, the Middle East, coastal India, and Central and South America. "True tilapias", a group of approximately 50 species of perch-like fishes, are endemic only to Africa and the Middle East. The group consists of three genera *Oreochromis, Sarotherodon*, and *Tilapia* (Trewavas, 1982). The objective of this study was to DNA barcode the cichlids available in Israel and compare them with *O. urolepis hornorum*.

Materials and Methods

The cichlid species were sampled from native waters (*Tilapia zillii, Tristramella simonis*, and *Astatotilapia flaviijosephi*), as well as from aquaculture and experimental facilities. Fin samples were collected from 104 individuals of six tilapiine species, two tilapia hybrid strains and two endemic wild cichlids (Table 1). The species *O. urolepis hornorum* is not available in Israel; thus, preserved fin samples from Brazil were used in this study. Florida red tilapia and Philippine red tilapia are commercially cultured hybrids.

DNA extraction was performed using the salting out procedure (Ma et al., 1996). DNA samples were diluted 1:10-1:50 and 3 µl of samples were used as a template for PCR amplification of the COI sequence. COI-3 primer cocktail and M13 primers were used for amplification and sequencing, respectively, following Ivanova et al. (2007). PCR products were run on 1.5% agarose gel. Relevant band sizes were excised from the gel, purified with DNA Montage Gel Extraction Kit (Millipore, Bedford, MA, USA), and sequenced. Sequence trace files were assembled using the GAP4 program (Staden et al., 1999). Amino-acid sequences were aligned with ClustalW using the default parameters and the GONNET weight matrix (http://clustalw.genome.jp/). Phylogenetic trees were constructed using MEGA (version 4.0.2) using the default models recommended by FISH-BOL bioinformatic tool for similar analyses.

Results and Discussion

All 104 examined samples were successfully amplified and sequenced. Sequence traces of COI 619 bp region were assembled, aligned, and compared. Sequence alignment may be generated using NCBI PopSet module (www.ncbi.nlm.nih.gov/sites/entrez?db=PopSet&cmd=search&term=209553463).

The COI sequences of the Florida and Philippine red tilapia hybrids were identical to those of *O. hornorum* and *O. aureus*, respectively, in agreement with documentation of the history of these strains (Watanabe et al., 1989, Galman and Avtalion, 1983, respectively).

Sequence comparison showed absence of intra-specific variability in the four commercially cultured species *O. aureus, S. galilaeus*, *O. mossambicus*, and *O. urolepis hornorum*. Two

Table 1. Israeli indigenous and introduced cichlid species and stocks barcoded in this project.

Species	*Origin of species*	*Origin of stock*	*Origin of sample*	*Sample size*
Sarotherodon				
S. galilaeus	Israel	Sea of Galilee	ARO, BIU	10
Oreochromis				
O. mossambicus	East Africa	Natal, South Africa	ARO	9
O. urolepis hornorum	North-East Africa	Brazil	CHESF	6
O. aureus	Egypt, Lower Nile	Univ. of Stirling, UK	ARO	5
O. aureus	Israel	Mehadrin Reservoir	ARO	7
O. aureus	Israel	Ein Feshkha	ARO	13
O. aureus	Israel	Ein Feshkha	BIU	4
O. aureus	Israel	Bet Shean Valley	Nir David	3
O. niloticus	Egypt	Univ. of Swansea, Wales	ARO	4
O. niloticus	Egypt, Lake Manzala (red mutant)	Univ. of Stirling, UK	ARO	3
O. niloticus	Egypt	Canada	ARO	2
O. niloticus	Ghana	Volta River	ARO, BIU	4
Florida red tilapia hybrid	Florida	Jamaica	Sde Eliyahu	6
Philippine red tilapia hybrid	-	Philippines	BIU	3
Tilapia				
T. zillii	Israel	Yarkon stream	BIU	7
T. zillii	Israel	Sea of Galilee	ARO	5
T. zillii	Israel	-	Gan Shmuel	4
Other cichlids				
Tristramella simonis	Israel	Jordan-Dead Sea rift valley	Sea of Galilee	3
Astatotilapia flaviijosephi	Israel	Jordan-Dead Sea rift valley	Sea of Galilee	6
Total				104

ARO = Agricultural Research Organization; BIU = Bar-Ilan University; CHESF = Fish Aquaculture Station, Brazil

sequence variants (FJ348104 and FJ348106) were detected in *O. niloticus* originating from Ghana, while only one variant (FJ348115) was detected in all other stocks originating from Egypt. In contrast to commercial species, 2-3 variants were detected in each of the non-commercial species *T. zillii, Tr. simonis*, and *A. flaviijosephi*. Analysis of intra-specific variation in *T. zillii* populations revealed two variants that differ by a single nucleotide substitution. Both variants were found in the Yarkon River (FJ348145 and FJ348147), but only one in the Sea of Galilee (FJ348140) and Gan Shmuel near Hadera stream (FJ348135).

Phylogenetic trees based on protein and DNA sequences are presented in Figs. 1a and 1b, respectively. Conventional taxonomy (Gittleman, 1981) predicted grouping the examined species into three groups: (1) *A. flaviijosephi*, (2) *Tr. simonis*, and (3) all other species that are the members of "true tilapias" and their sub-groups i.e. *Oreochromis, Sarotherodon*, and *Tilapia*. The protein phylogenetic tree (Fig. 1a) fits the expected conventional taxonomy; "true tilapias" are grouped in the same branch due to identical amino-acid sequences. However, the DNA-based tree (Fig. 1b) does not fit this taxonomy. In general, the same phylogenetic tree was obtained whether the third codon nucleotide or the whole nucleotide sequence was compared. This tree has three major branches: (1) *T. zillii*; (2) *A. flaviijosephi*; and (3) all other species including *Tr. simonis*. The latter group is sub-grouped into two major branches: (1) three species from the Sea of Galilee and (2) three African *Oreochromis* species. Thus, two major findings were unexpected: first - *O. aureus* clusters with *S. galilaeus* and *Tr. simonis*, according to the common geography, but not with the other members of the *Oreochromis* genus; and second - *T. zillii* is distant from other "true tilapias", even more than *A. flaviijosephi*.

In accordance with Russo et al. (1996), we conclude that the protein sequence rather than the DNA sequence represents the more likely phylogenetic tree. However, the protein sequence has low phylogenetic resolution for evolutionary-young species such as the Tilapiini, in which case a DNA-based tree might be advantageous. The convergence of the DNA sequences of the three taxonomically distant species from the Sea of Galilee (*O. aureus*, *S. galilaeus*, and *Tr. simonis*) into a single branch (Fig. 1b) is rather surprising and should be further studied.

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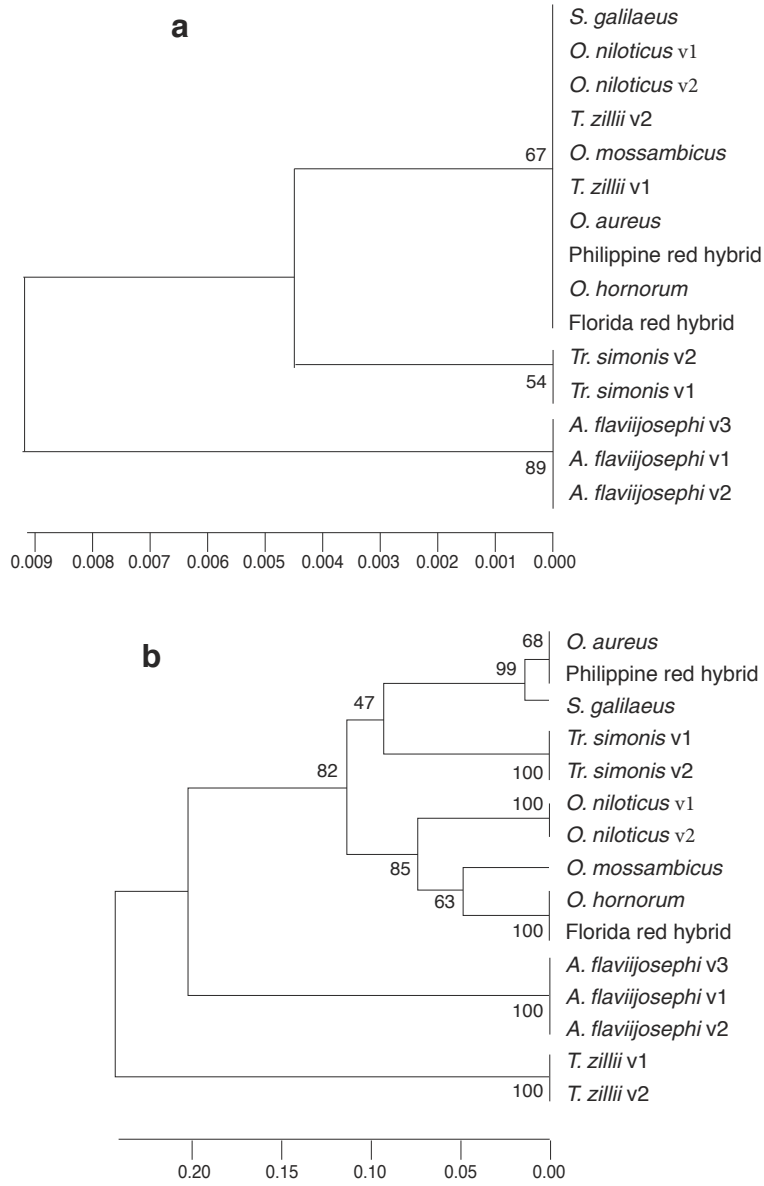

Fig. 1. Phylogenetic trees of endemic and introduced cichlid species. Distances were calculated using either (a) amino-acids translation of the 619 bp region of COI mitochondrial gene and the Dayhoff Matrix model, or (b) the third nucleotide in codons present in the 619 bp region of COI mitochondrial gene and the Kimura 2-parameter model. The trees were generated by MEGA4 using the neighbor-joining method and bootstrap (500 replicates) analysis. Numbers at tree junctions indicate the percentage of trees that correspond to the consensus bootstrap tree. The scale on the X-axis represents the distance in number of (a) amino-acid or (b) nucleotide substitutions per site. v = variant.

References

Galman O.R. and Avtalion R.R., 1983. A preliminary investigation of the characteristics of red tilapias from the Philippines and Taiwan. pp. 291-301. In: L. Fishelson, Z. Yaron (Comps.). *Proc. Int. Symp. Tilapia in Aquac.*, May 8-13, Nazareth, Israel. Tel Aviv Univ., Ramat Aviv, Israel.

Gittleman J.L., 1981. The phylogeny of parental care in fishes. *Anim. Behav.*, 29:936-941.

Hebert P.D.N., Cywinska A., Ball S.L. and J.R. de Waard, 2003. Biological identifications through DNA barcodes. *Proc. Royal Soc. B: Biol. Sci.*, 270:313-321.

Ivanova N.V., Zemlak T.S., Hanner R.H. and P.D.N. Hebert, 2007. Universal primer cocktails for fish DNA barcoding. *Mol. Ecol. Notes,* 7:544-548.

Ma R.Z., Beever J.E., Da Y., Green C.A., Russ I., Park C., Heyen D.W., Everts R.E., Fisher S.R., Overton K.M., Teale A.J., Kemp S.J., Hines H.C., Gurin G. and H.A. Lewin, 1996. A male linkage map of the cattle (*Bos taurus*) genome. *J. Hered.*, 87:261-271.

Russo C., Takezaki N. and Nei M., 1996. Efficiencies of different genes and different tree-building methods in recovering a known vertebrate phylogeny. *Mol. Biol. Evol.*, 13:525-536.

Staden R., Beal K.F. and J.K. Bonfield, 1999. The Staden package, 1998. *Methods Mol. Biol.,* 132:115-130.

Trewavas E., 1982. Generic groupings of Tilapiini used in aquaculture. *Aquaculture*, 27:79-81.

Ward R.D., Zemlak T.S., Innes B.H., Last P.R. and P.D.N. Hebert, 2005. DNA barcoding Australia's fish species. *Phil. Trans. Royal Soc. London,* Ser. B, Biol. Sci., 360:1847-1857.

Watanabe W.O., Ernst D.H., Olla B.L. and R.I. Wicklund, 1989. Aquaculture of red tilapia (*Oreochromis* sp.) in marine environments - state of the art. *AQUACOP IFREMER Actes Colloq.*, 9:487-498.