#### **DNA-Based Identifications of Tilapia in Hawaii**

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E.EA.O.K

# A local Tilapia



#### **Tilapia Aquaculture**

High-quality food fish; Easy to reproduce, breed and farm; > Farming for freshwater and brackish water;  $\triangleright$  Excellent species for aquaponics; > A potential growth area for Hawaii with groundwork having been laid in the marketing area.

### Challenges of Tilapia Farming in Hawaii

Slow growth performance

Impossibility of importing and using known fast growing strains and species.

Questions about the potential of local genetic resources. Are there good genetic resources here?

Disease including PRLO.

#### The Goal and Objectives

- \* The long-term goal of the project is to have fast-growing tilapia for farmers in Hawaii.
- \* Main Objectives
- 1. Identification of the tilapia strains and hybrids existing in the wild and captive populations by DNA barcoding method
- 2. Successfully allow importation of fast growing strains <u>or</u> develop DNA-based testing tools for selection of high-growth tilapia by selectively breeding local tilapia resources.

What is DNA and genome?
Permanent part of the body cells, inheritable;
Genome: a whole collection of the DNA of a species;
Different species are made of different genomes;
Strains with one species have slightly different DNA sequences.

Genes

Cell Nucleus Containing 23 Pairs of Chromosomes

Chromosomes

Bases

**DNA Strand** 

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# How Can DNA-based Technology be Helpful for Tilapia Farming?

Accurate technologies are available in amplification of DNA and obtaining sequence information.

Variations in DNA sequence are present in individuals, stains, hybrids in all tilapia species.

Quality and inexpensive technology by DNA barcoding and microsatellite DNA markers can be used for tilapia strain/species identifications and growth trait selection.

# **DNA Barcoding**

- It is DNA sequence-based taxonomic method of species identification.
- A 648 base-pair region in the mitochondrial (maternal) cytochrome c oxidase 1 gene (COI) has been extensively studied. FISH-BOL database. Obtain sequences of known species as niloticus, mossambicus, aureus and so on.
- The DNA sequence COI can identify parents (mothers) of existing species in Hawaii, including niloticus.

- Amplify and sequence DNA of available tilapia in Hawaii.
- If we have niloticus here already, DOA may let us import.
- If we have niloticus here already, we can reverse breed to get back a fast growing strain.
- (These methods have been used to identified mislabeled fillets.)















#### **DNA** Extraction









ca	A-TRACOMPTARATACCACTORACOATACTTTAROACCORACACRACT-C	194
O. BALOYO. MONSAATE33481	A-TAACHAFTAAATHOCACTGAACGATAGYTTAAGACGBAACACAACT-C	196
8	A ARACATTTCALARCCATTARACOACROTTLANACOARACACARCT-C	1.95
O. BORRADIOAY033459	A AAACAPTPCAAAAOCACTAAACOACAOPTTAAOAOODAACACAACT-C	195
<b>0</b>	A AAACATTTCAAAACCACTAAACGACADTTTAAGACCBAACACAACT-C	195
P	A AAACATTTAAAAACCACTGAACGACAGTTAAGACCGAACACAACT -C	197
0. wrolepisAF296467	A - AAA/CATETAAAAAOCACTOAACOACADTTAADACOBAACACAACT-C	197
A	II-TAAATTTUALAAAOCACTUAACUACA-TTTAABAOCUAACACAAOCAC	1.90
0 americal/220051	B. TARATTTORAGAA CONCECTORS CONCELL TITLEDA CONSECUCIÓN	100
8	O-TABATTTOAAAAAOCACTOAACOACA TTTAADACODAACACAAOCAC	1.90
CI	G-TAAATTTOAAAAAOCACTGAACGACA-TTTAAGAOCGAACACAAOCAC	199
E	B-TALATTTORAGAAACTACTOAACOACA.TTTAAOACOBAACACBACCAC	190
H house the contract state of the second st	A TAL TTTOALAAACCACTOAACDACL TTTAADACCBAACACAACCAT	197
0.8410t10WAF485083	A-TAR-TTTGAAAAAOCACTGAAGGACA-TTTAAGACGGAACACAAGCAC	197
I	ANTANTOTTCARANTOCHOTAANCOATANCOTARGACODARCHOANTCHO	197
f. melanotheronAF404717	ARTARTOTTCALARTCCACTARROBATARCCTARRACCRARCACARTCRC	197
7 renialliaF220054	A CATATTTTATAAANTACTAAACDATANTTTAAGACOBADCACDATAAC	195
0	A CATAOCTTATAAANTACTAAACOATAOTTTAADACOBADCACAATAAC	195



DNA Sequence Alignment and Recognize



A-TAACGATTAAATACCACTGAACGATAGTTTAAGACCGAACACAACT-C	196
A-TAACGATTAAATACCACTGAACGATAGTTTAAGACCGAACACAACT-C	196
A-AAACATTTCAAAACCACTAAACGACAGTTTAAGACCGAACACAACT-C	195
A-AAACATTTCAAAACCACTAAACBACASTTTAABACCBAACACAACT-C	195
A-AAACATTTCAAAACCACTAAACGACAGTTTAAGACCGAACACAACT-C	195
A-AAACATTTAAAAAOCACTGAACGACAGTTTAAGACCGAACACAACT-C	197
A-AAACATTTAAAAACCACTGAACGACAGTTTAAGACCGAACACAACT-C	197
G-TAAATTTGAAAAACCACTGAACGACA-TTTAAGACCGAACACAACCAC	198
9-TAAATTTGAAAAACCACTGAACGACA-TTTAAGACCGAACACAACCAT	198
G-TAAATTTGAAAAACCACTGAACGACA-TTTAAGACCGAACACAACCAC	198
G-TAAATTTGAAAAACCACTGAACGACA-TTTAAGACCGAACACAACCAC	198
9-TAAATTTGAAAAACCACTGAACGACA-TTTAAGACCGAACACAACCAC	198
A-TAA-TTTGAAAAACCACTGAACGACA-TTTAAGACCGAACACAACCAT	197
A-TAA-TTTGAAAAACCACTGAACGACA-TTTAAGACCGAACACAACCAC	197
AATAATCTTCAAAATCCACTAAACGATAACCTAAGACCGAACACAATCAC	197
AATAATCTTCAAAATCCACTAAACBATAACCTAABACCBAACACAATCAC	197
A-CATATTTTATAAAGTACTAAACGATAGTTTAAGACCGAGCACGATAAC	195
A-CATACCTTATAAA9TACTAAACBATA9TTTAA9ACC9A9CACAATAAC	195
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Population (Site)	Number of fish	Claimed species	Genetic composition
	Aquaculture Sites		
А	51	Unknown	O. aureus
в	34	O. Aureus	O. aureus
С	30	Unknown	O. niloticus × O. mossambicus and O.aureus
D	102	Unknown	O. mossambius
Е	50	Unknown	O. aureus
F	50	Unknown	O.urolepis
G	50	Unknown	0.mossambicus
Н	29	Unknown	O. niloticus
	Wild/Feral Sites		
	44	Unknown	S. melanotheron
J	44	Unknown	T.rendali
K	22	unknown O. nilotic	us x O. mossambicus

#### Table 1. Identifications of the tilapia samples by DNA sequence.

### Summary

- Based on 382 samples collected from 14 farm (captive) and wild tilapia populations in Oahu and the Hawaii Islands, mtDNA CR had higher nucleotide diversity than COI.
- The neighbor-joining tree analysis identified seven distinctive tilapia species: O. aureus, O. mossambicus, O. niloticus, S. melanotheron, O. urolepies, T. redalli, and a hybrid of O. massambicus and O. niloticus.

## Significances

- Determination of genetic resources in Hawaii
  - To allow importation of fast growing species since they may already be here or
  - To develop genetic selection programs to fast growing strains that originally got in
- To use microsatellite markers if there is a selected breeding program.
- Sponsored by CTSA.