# COMPARATIVE ANALYSIS OF MICRORNAs TRANSCRIPTOME EXPRESSION IN CHITRALADA, RED STIRLING AND IN CROSSBRED NILE TILAPIA (*Oreochromis niloticus*) USING HIGH THROUGHPUT SEQUENCING

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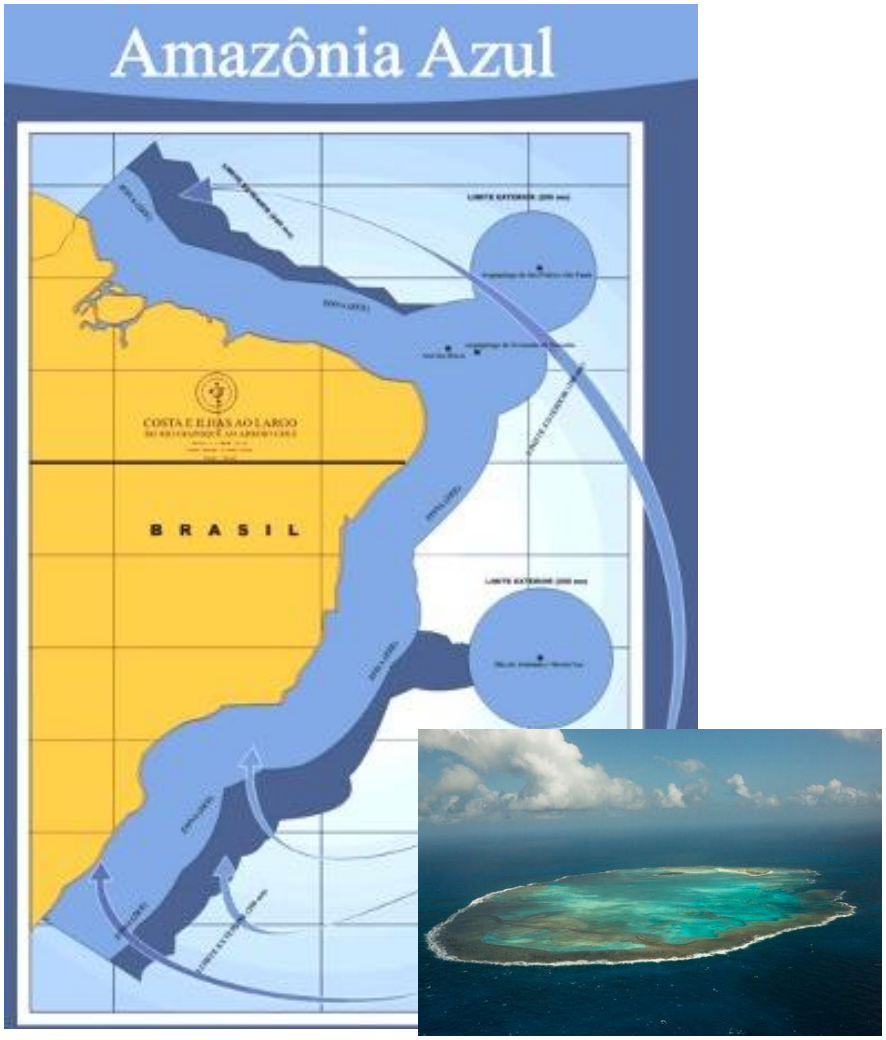
# Agenda

- Fisheries and Aquaculture
- Nile tilapia crossbreed and microRNAs
- Workflow
- Main findings

# Overview



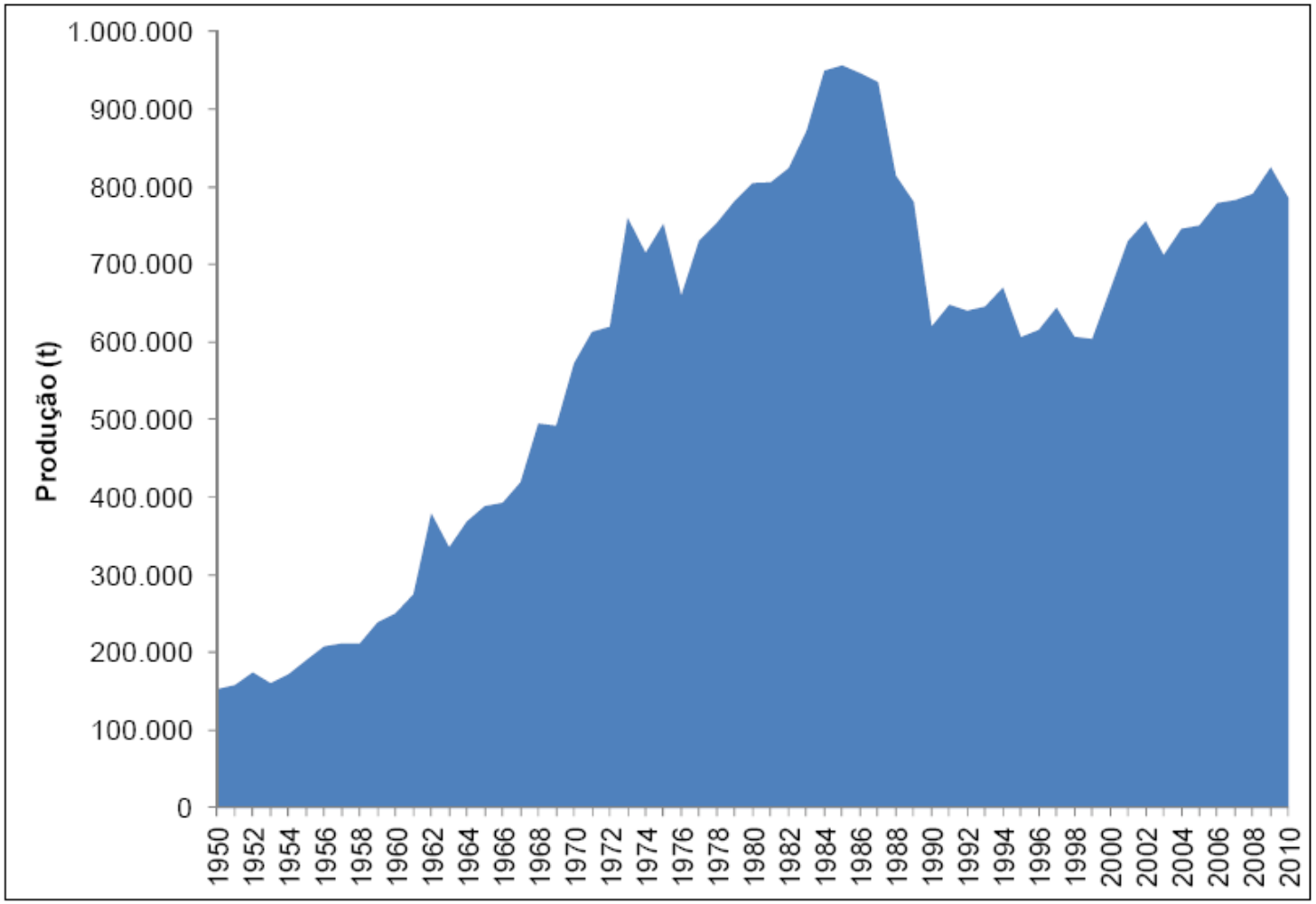

✓ 12.951.766 Km² of coast

✓12% world freshwater

# Fisheries and Aquaculture

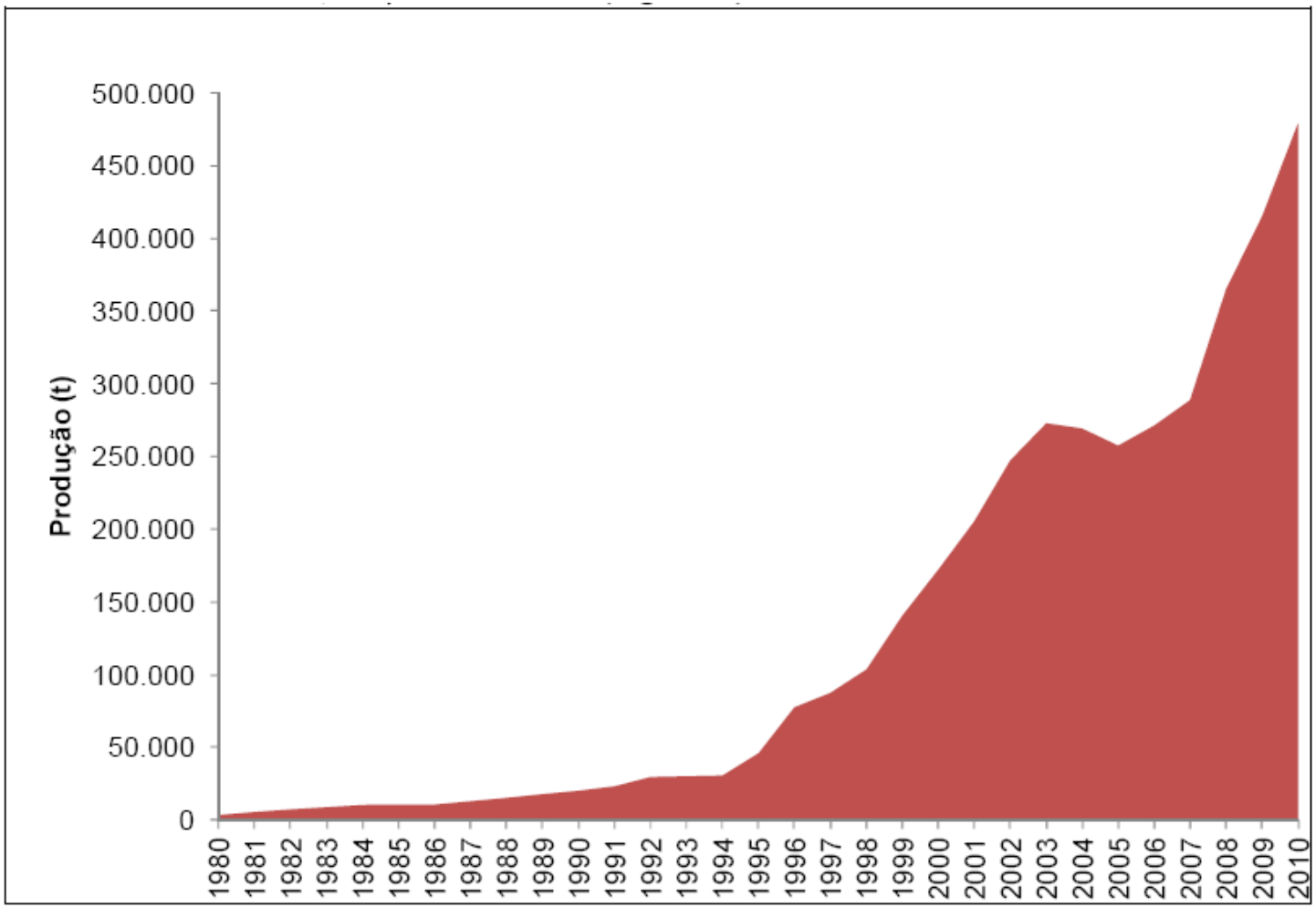
- ✓ Brasil nowadays: 1.2 million ton/year
- ✓ Ministry of Fisheries and Aquaculture
- ✓ 2008 – plan “Improving Fisheries and Aquaculture” – aim to increase by 40% total production

# Fisheries




Fisheries production (marine and continental) 1950-2010

# Aquaculture




Aquaculture production (marine and continental) 1950-2010

# Nile tilapia (*Oreochromis niloticus)*

- Top farmed freshwater fish
- 40% - 133,000 tons/year
- Genome sequenced
- Genetically improved
- Breeds and lines were developed

Chitralada

Red Stirling

# Introgressive crossbreeding

Chitralada X Red Stirling

F1 (50% Chitralada)

Chitralada X F1 (Red)

RC1 (75% Chitralada)

Chitralad X RC1 (Red)

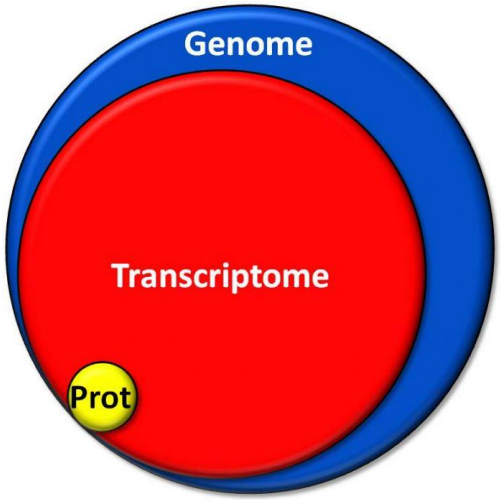
RC2 (87,5% Chitralada)

**Heterotic crossbreed**

Hilsdorf, 2013

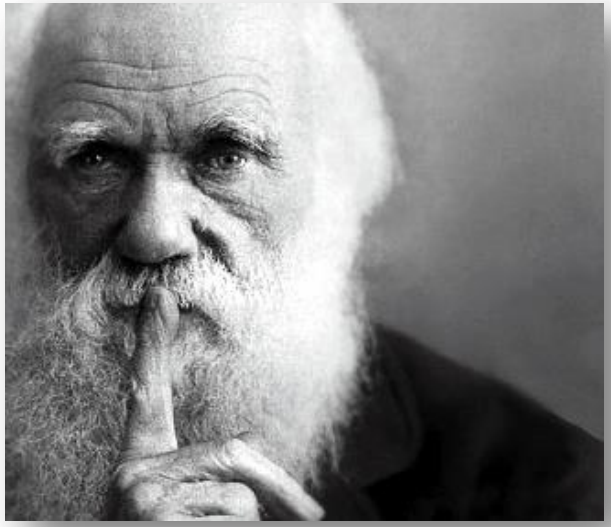
# Main question

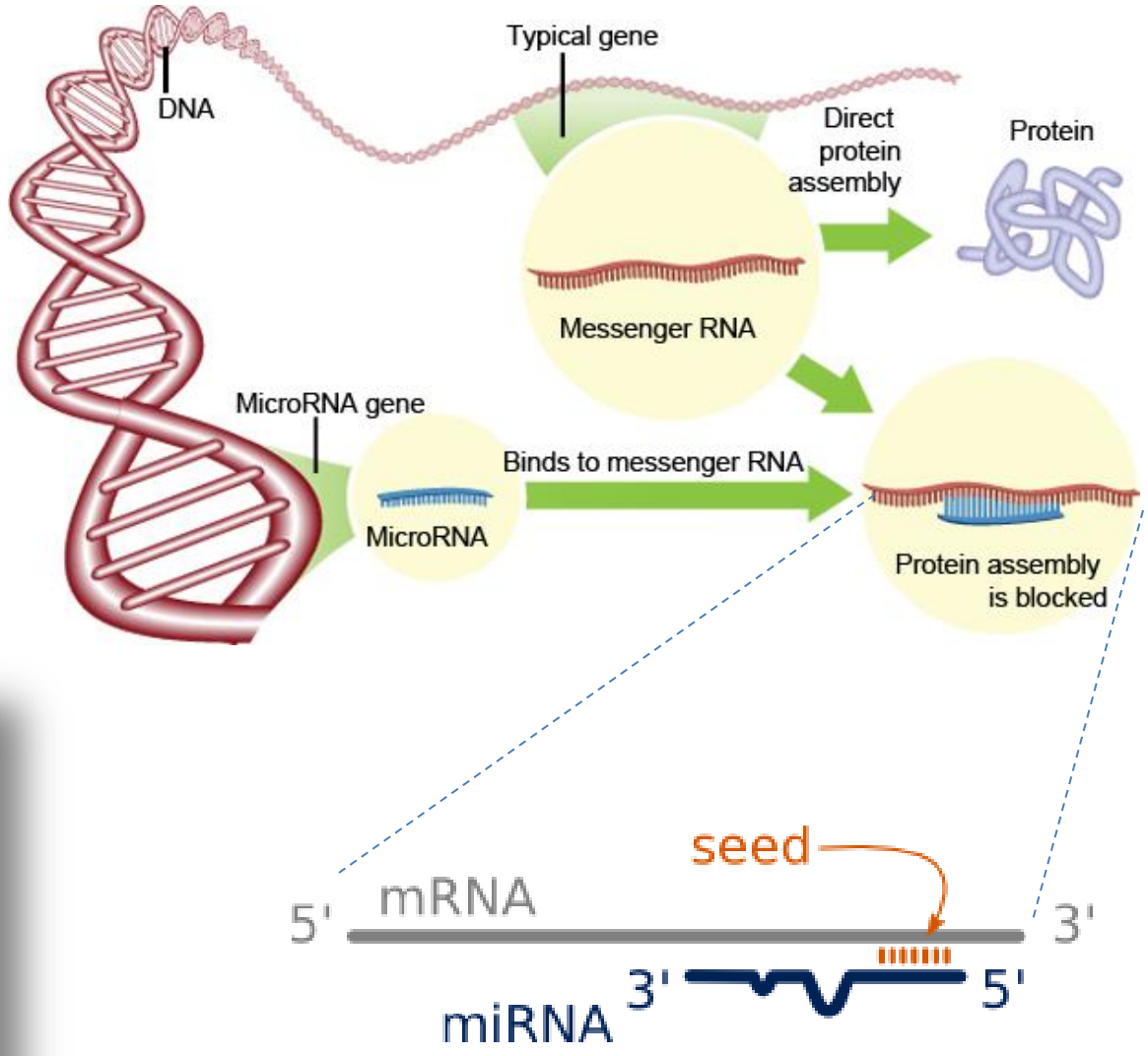
- What are the molecular mechanisms underlying heterosis?
  - DNA – SNPs; QTLs
  - RNA – variable gene expression
  - Proteins




**ncRNAs: microRNAs**

- ✓Transcriptome
- ✓MicroRNAs

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# Tilapia microRNAs

NATURE | ARTICLE OPEN

日本語要約

The genomic substrate for adaptive radiation in African cichlid fish

David Brawand, Catherine E. Wagner, Yang I. Li, Milan Malinsky, Irene Keller, Shaohua

Molecular Biology Reports
August 2014, Volume 41, Issue 8, pp 4953-4963

Date: 22 Apr 2014

MicroRNA repertoire for functional genome research in tilapia identified by deep sequencing

Biao Yan, Zhen-Hua Wang, Chang-Dong Zhu, Jin-Tao Guo, Jin-Liang Zhao

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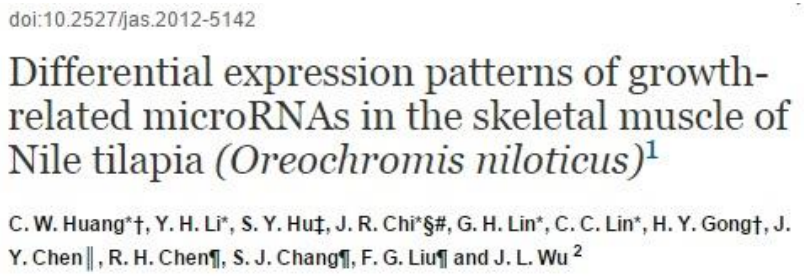
RESEARCH ARTICLE

Identification and Characterization of MicroRNAs in Ovary and Testis of Nile Tilapia (*Oreochromis niloticus*) by Using Solexa Sequencing Technology

Jun Xiao, Huan Zhong, Yi Zhou, Fan Yu, Yun Gao, Yongju Luo, Zhanyang Tang, Zhongbao Guo, Enyan Guo, Xi Gan, Ming Zhang, Yaping Zhang

Published: January 23, 2014 • DOI: 10.1371/journal.pone.0086821



doi:10.2527/jas.2012-5142

Differential expression patterns of growth-related microRNAs in the skeletal muscle of Nile tilapia (*Oreochromis niloticus*)[1]

C. W. Huang*†, Y. H. Li*, S. Y. Hu‡, J. R. Chi*§#, G. H. Lin*, C. C. Lin*, H. Y. Gong†, J. Y. Chen‖, R. H. Chen¶, S. J. Chang¶, F. G. Liu¶ and J. L. Wu[2]

Still need to be better addressed:

Total miRNA composition in Nile tilapia

miRNAs roles in growth

miRNA roles in the crossbreed heterosis phenotype (if any)

# Objective

- Characterize the miRNA expression profile in Nile tilapia

- Compare miRNA signatures between crossbreed and parentals (lines)

# Material and Methods

Embryos

Adults

RNA extraction

HiSeq 2000 Illumina

Bioinformatics → **miRProf (The UEA small RNA Workbench)**

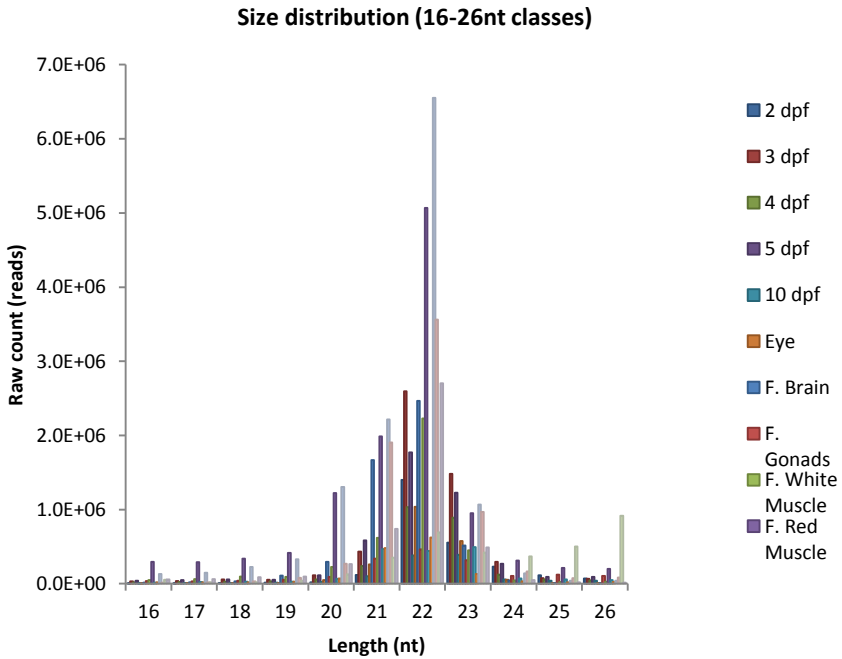
miRNA characterization

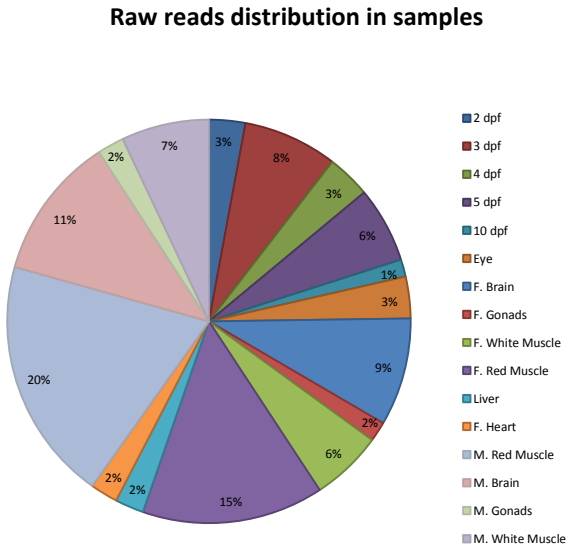
**- muscle**, gonads, brain, gill, skin, kidney, splen, liver, heart

# Results

## Read count numbers generated by global miRNA sequencing

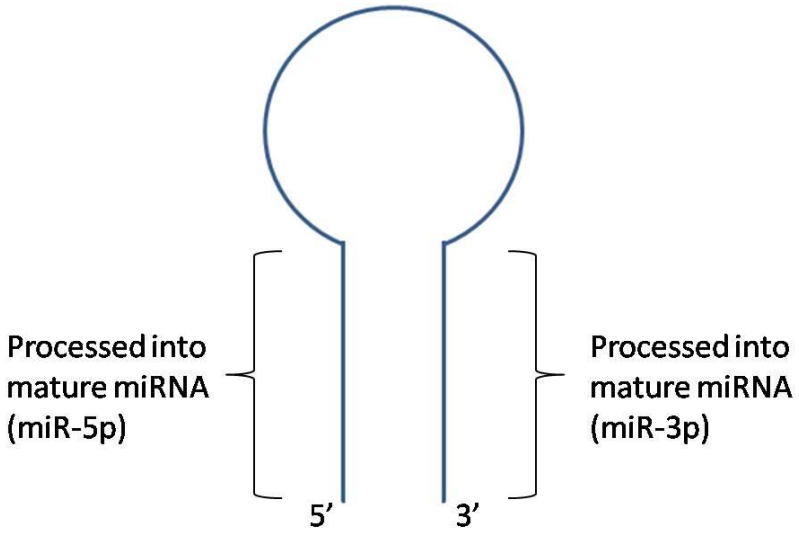
| Reads | Counts |
|---|---|
| Total | 284.734.849 |
| Sized between 15 and 26nt | 116.816.138 |
| 100% genome similarity | 90.596.359 |
| Unique | 5.519.754 |



# Results

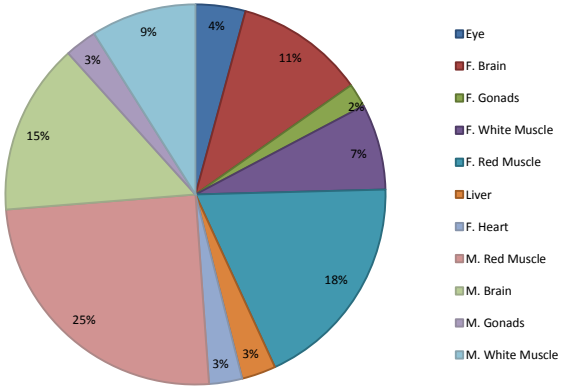
- 271 pre-miRNAs could be traced to genomic locations representing 314 miRBase known mature miRNAs
- Mature miRNAs were:
  - 195 miRNAs-5p
  - 206 miRNAs-3p
- Grouped into 234 families



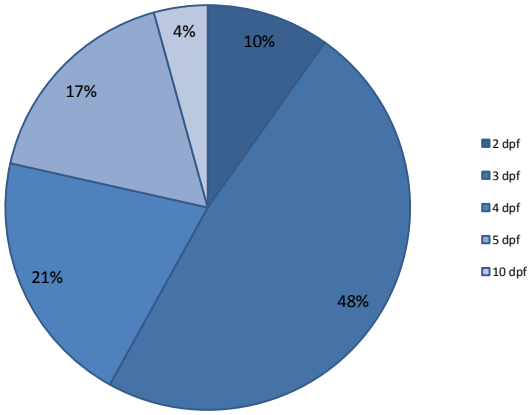

- Also several isoforms (isomiRs) were identified

# Results

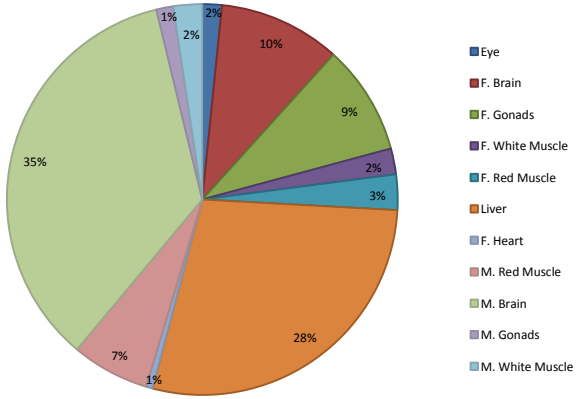
**Raw reads distribution in tissues from known miRBase miRNAs**




**Raw reads distribution in embryo stage from novel miRNAs**




**Raw reads distributions in tissues from novel miRNAs**



# Results

**List of the top 10 highly expressed miRNAs in *O. niloticus*.**

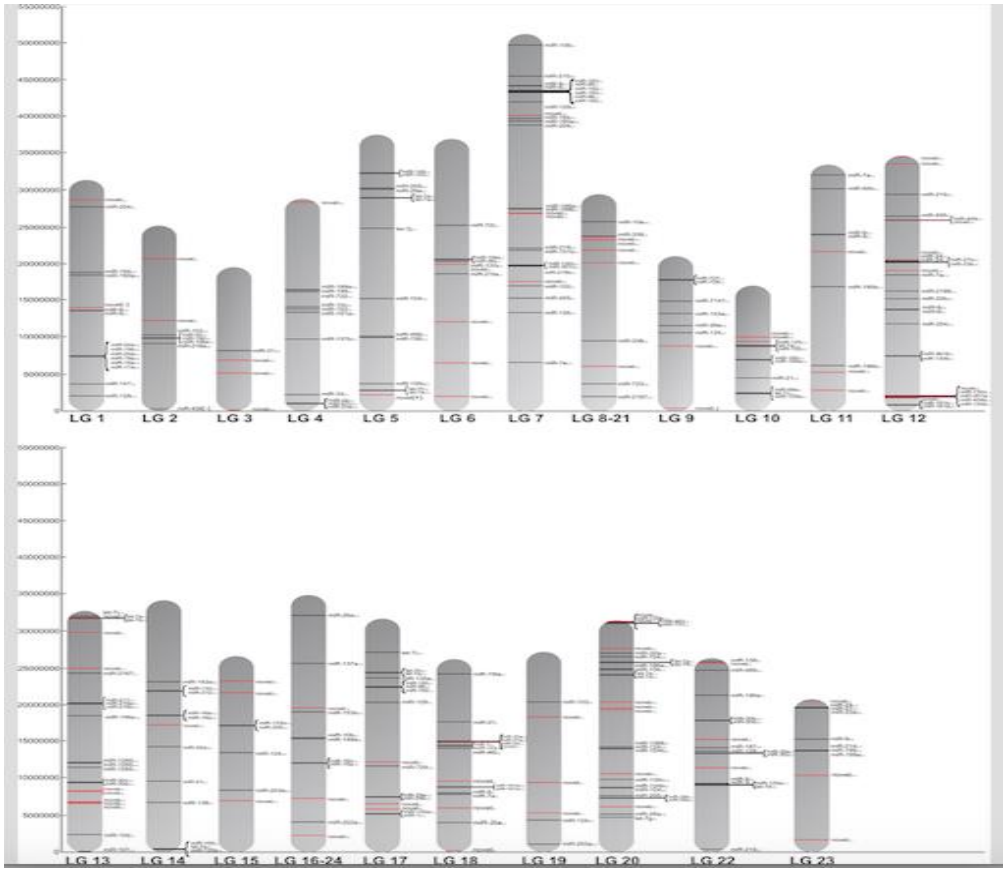
| Eyes | | liver | | heart | | red muscle(female) | | white muscle (female) | |
|---|---|---|---|---|---|---|---|---|---|
| mir-183 | 544000 | mir-122 | 363000 | mir-126 | 224000 | mir-10 | 175000 | mir-26 | 122000 |
| mir-182 | 65900 | let-7 | 60200 | mir-100 | 163000 | mir-100 | 114000 | let-7 | 106000 |
| let-7 | 57100 | mir-100 | 58300 | let-7 | 93300 | mir-26 | 90800 | mir-10 | 105000 |
| mir-100 | 53400 | mir-199 | 53000 | mir-99 | 91400 | let-7 | 88100 | mir-1 | 105000 |
| mir-96 | 49900 | mir-26 | 32800 | mir-26 | 87800 | mir-143 | 36600 | mir-206 | 60000 |
| mir-181 | 33200 | mir-126 | 30600 | mir-27 | 41400 | mir-126 | 31700 | mir-100 | 57300 |
| mir-184 | 24900 | mir-192 | 30300 | mir-499 | 38900 | mir-27 | 27500 | mir-125 | 54000 |
| mir-99 | 19000 | mir-181 | 19900 | mir-1 | 26800 | mir-30 | 27300 | mir-133 | 47100 |
| mir-124 | 12500 | mir-30 | 19400 | mir-30 | 20500 | mir-181 | 25000 | mir-21 | 28900 |
| mir-146 | 8240,88 | mir-146 | 17700 | mir-21 | 20400 | mir-99 | 21800 | mir-199 | 23900 |
| mir-26 | 5899,12 | mir-99 | 10900 | mir-125 | 19200 | mir-21 | 19300 | mir-22 | 20400 |

# Results

**List of the top 10 highly expressed miRNAs in *O. niloticus*.**

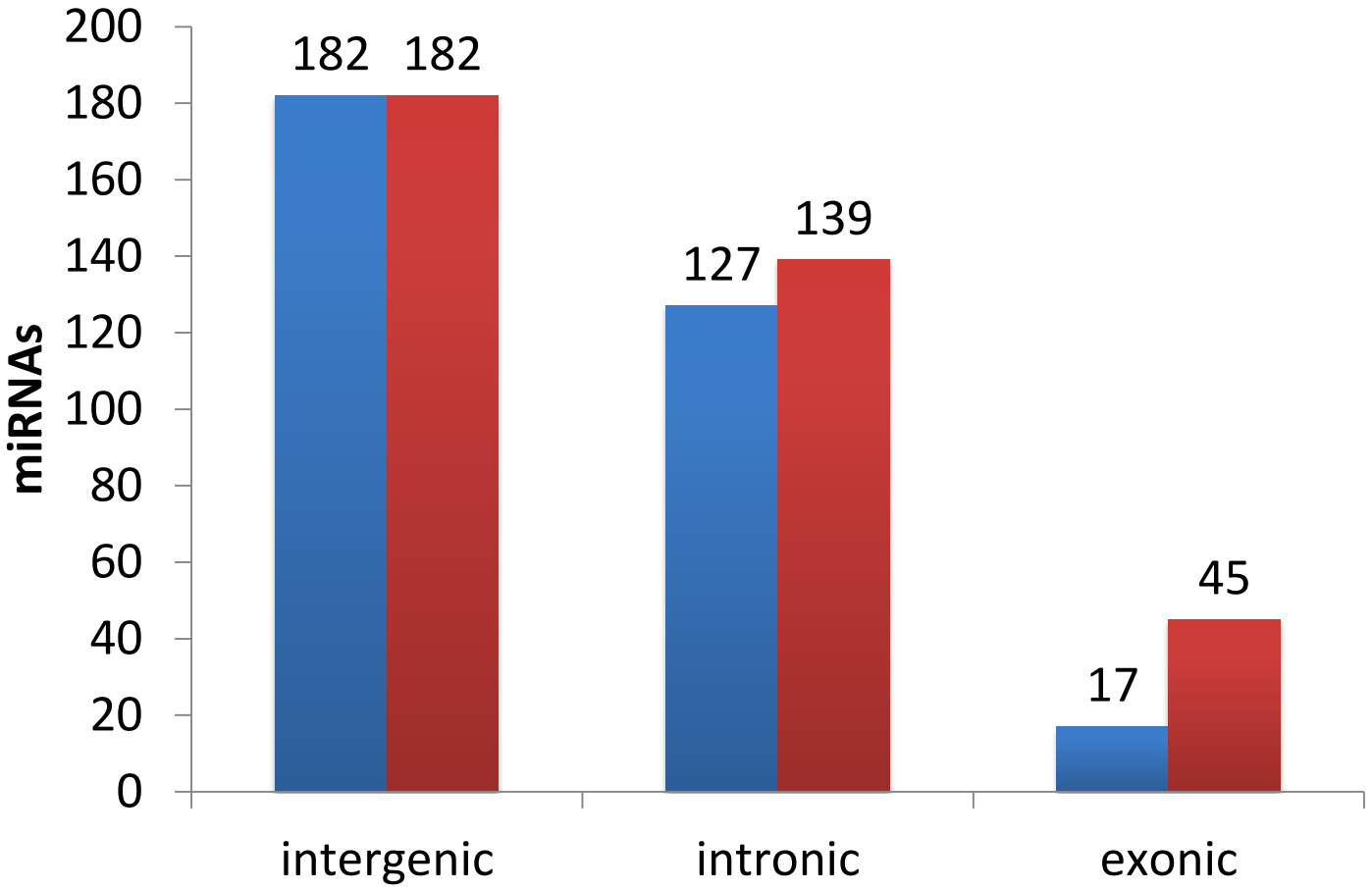
| red muscle (male) | | white muscle (male) | | gonads | | Testis | | brain (female) | |
|---|---|---|---|---|---|---|---|---|---|
| mir-10 | 209000 | mir-10 | 208000 | mir-100 | 143000 | mir-10 | 79900 | mir-100 | 253000 |
| mir-100 | 200000 | mir-1 | 147000 | let-7 | 107000 | mir-100 | 66300 | mir-125 | 150000 |
| mir-26 | 102000 | let-7 | 81900 | mir-146 | 107000 | mir-143 | 45700 | let-7 | 149000 |
| let-7 | 68900 | mir-100 | 72700 | mir-21 | 41800 | mir-26 | 43600 | mir-26 | 79500 |
| mir-181 | 54200 | mir-26 | 69200 | mir-143 | 39100 | mir-126 | 41300 | mir-99 | 68700 |
| mir-125 | 43900 | mir-206 | 63400 | mir-27 | 27400 | let-7 | 30000 | mir-126 | 37500 |
| mir-99 | 37700 | mir-133 | 45100 | mir-10 | 26800 | mir-30 | 23700 | mir-9 | 33600 |
| mir-143 | 36900 | mir-21 | 39500 | mir-126 | 22500 | mir-146 | 21400 | mir-124 | 26200 |
| mir-30 | 28000 | mir-99 | 18900 | mir-99 | 12900 | mir-7 | 19400 | mir-10 | 24500 |
| mir-126 | 23800 | mir-199 | 15000 | mir-22 | 5892,45 | mir-21 | 17700 | mir-128 | 20500 |
| mir-27 | 22100 | mir-126 | 14600 | mir-7 | 5890,49 | mir-22 | 14600 | mir-27 | 14800 |

# Results




**miRNAs distribution in Nile tilapia genome (linkage groups)**

# Results


200
180
160
140
120
100
80
60
40
20
0
miRNAs
182
182
127
139
17
45
intergenic
intronic
exonic

# Results

- ↓ Crossbreed (Red Stirling)
  - 13 miRNAs
- ↑ Crossbreed (Chitralada)
  - 4 miRNAs

# Results

## Low expressed in Crossbreed in comparison to Red Stirling

| miRNA | H5 | H6 | R5 | R6 | R5/ H5 | R6 /H6 |
|---|---|---|---|---|---|---|
| let-7e-3p | 0,77 | 0,14 | 1,23 | 0,63 | 0,67 | 2,12 |
| mir-16-3p | 1,03 | 0,58 | 0,98 | 4,08 | -0,25 | 1,26 |
| mir-24-5p | 1,03 | 2,32 | 5,4 | 6,44 | 2,4 | 1,47 |
| mir-122-5p | 40,01 | 2356,2 | 609,95 | 2317,05 | 3,93 | -0,02 |
| mir-124-3p | 0,51 | 1,88 | 0,57 | 13,67 | 0,16 | 2,86 |
| mir-135a-5p | 0,77 | 0,43 | 0,25 | 3,30 | -1,65 | 2,92 |
| mir-153-3p | 0,51 | 4,15 | 2,29 | 8,48 | 2,16 | 1,03 |
| mir-192-5p | 17,44 | 1008,3 | 215,10 | 1171,56 | 3,62 | 0,21 |
| mir-194-5p | 1,54 | 23,74 | 7,86 | 44,61 | 2,35 | 0,91 |
| mir-216b-3p | 0,00 | 0,58 | 0,98 | 3,14 | 0 | 2,44 |
| mir-219-5p | 0,00 | 0,14 | 0,00 | 0,63 | 0 | 2,12 |
| mir-301-5p | 0,51 | 6,37 | 3,44 | 5,03 | 2,74 | -0,34 |
| mir-458-5p | 0,51 | 6,66 | 3,93 | 5,66 | 2,94 | -0,24 |

# Results

**High expressed in Crossbreed in comparison to Chitralada**

| miRNA | H5 | H6 | C5 | C6 | C5/H5 | C6/H6 |
|---|---|---|---|---|---|---|
| mir-124-3p | 0,51 | 1,88 | 0,32 | 0,26 | -0,68 | -2,86 |
| mir-219-5p | 9,23 | 24,03 | 1,44 | 24,92 | -2,68 | 0,05 |
| mir-301-3p | 1,03 | 0,87 | 0,24 | 0,32 | -2,1 | -1,42 |
| mir-458-5p | 0,51 | 0,14 | 0,12 | 0,19 | -2,1 | 0,43 |

# Results

- Among these miRNAs
  - let-7 has been previously enrolled in fish growth
  - mir-122 - pigs

# Results

- let-7 (let-7a-3p)
  - GH-2 (Barozai, 2012)

Gene 499 (2012) 163–168

Contents lists available at SciVerse ScienceDirect

Gene

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Short communication

## Identification and characterization of the microRNAs and their targets in *Salmo salar*

Muhammad Younas Khan Barozai *

*Department of Botany, University of Baluchistan, Sariab Road, Quetta, Pakistan*

# Results

- let-7
  - Myostatin (De Santis et al., 2008)

**BMC Genomics**

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Research article

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**Molecular characterization, tissue expression and sequence variability of the barramundi (*Lates calcarifer*) myostatin gene**

Christian De Santis, Brad S Evans, Carolyn Smith-Keune and Dean R Jerry*

Address: Aquaculture Genetics Research Program, School of Marine and Tropical Biology, James Cook University, Townsville, Queensland, 4811, Australia

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* Corresponding author

# Results

- miR-122
  - Fat deposition – pigs (Chen et al., 2012)

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PLoS one

## Solexa Sequencing Identification of Conserved and Novel microRNAs in Backfat of Large White and Chinese Meishan Pigs

Chen Chen[1], Bing Deng[1], Mu Qiao[1], Rong Zheng[1], Jin Chai[1], Yi Ding[1], Jian Peng[2]*, Siwen Jiang[1]*

1 Key Laboratory of Swine Genetics and Breeding of Agricultural Ministry, and Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, People's Republic of China, 2 Department of Animal Nutrition and Feed Science, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, People's Republic of China

# Summary

- Several novel miRNAs candidates were identified
- Prevalence of miRNA-3p in some tissues is indicative of arm-shifting
- Differentally expressed miRNAs in Crossbreed individuals may support their phenotype

# Research group

Dr. Danillo Pinhal

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Dr. Marco Dias

Marcus Frohme
(Germany)

Simon Moxon
(UK)

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