# Diversification of Marine Aquaculture through Genomics: applications to breeding programs in novel species

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### Why genomics at the initiation of a breeding program?

- Marine aquaculture is a diverse sector with a broad range of species
  - Initial broodstock sourced from the wild (possible bottlenecks)
    - Few generations in culture (not many generations in captivity)
    - Breeding system and life cycle often not well-characterized
  - Variable grow-out environments RAS vs Net cages



# **Genetic issues in new programs**

- Population structure
  - Endemic distribution and possible outbreeding depression
  - Conflicts between aquaculture and wild populations.

- Sex determination in early life history stages
  - Broodstock management
- Genotype –phenotype relationship
  - trait variation and breeding values

- Small founding population
  - Effective population size
- Reproductive system unknown
  - Influences on effective population size and inbreeding
  - Assymetric parental distribution



### Example: Seriola lalandi



Proposed Seriola species, based on phylogeny developed by Martinez-Takeshita et al. 2015



# Example: Seriola lalandi



- Programa de Diversificación de la acuicultura Chilena
  - Development of new industries based on marine aquaculture
  - Commercialization of species with high export potential.
  - Private-Public funding program













## **Part I: Development of Genomic resources**

- Draft Genome assembly
- SNP Genotyping chip that captures the geographic variation in *Seriola*
- SNP Validation based on national and international populations
- RNAseq for functional annotation of traits of interest
- Sex determination

### **Genome Reference assembly**



Male and Female randomly selected from the broodstock

#### Illumina sequencing

- Two Mate pair libaries
- per sex (60x)
- Two pair-end libraries per sex (70x)
- For each sex

#### Masurca assembly

Cabog (Contig to super scaffold)

## **Genome reference assembly**

- Draft genome:
  - Total size: 662.5Mb; representing 95% of the genome.
  - N50: 2n 403,974bp
  - GC (%): 40.8%
  - Contigs > 5000bp = 5,900



# **SNP discovery**

#### Sex specific Sequence data (17 males Map to reference sequence and SNP and 17 females at 9x) genome Discovery (Freebayes) **Sex locus GWAS** and Fst **SNP** array determination analysis (PLINK) (90 k)

#### **Sex determination**

- Evidence of ZW / ZZ sex determination
- Conducted genome re-sequencing of 17 males and 17 females
- Genome coverage approx. 9x
- GWAS based on genome-wide sequence data

## **GWAS for sex determination: genome assembly**

**450 K SNPS** 



#### **Genome annotation**



# Heterozygosity across sex-determining gene

Position, bp

286000 286500 287000 287500 288000 288500 289000 289500 290000



Validated SNPs using High Resolution Melting of sexed adults

# **Pathway: Steroid Hormone Biosynthesis**

• pre-receptor regulation of steroid hormone action (Mindnich et al. 2003, Prehn et al 2009)



• First evidence of the role of a gene known to regulate steroid production in sex determination (Martinez, et al., 2019. In preparation)

# **Development of the SNP chip**

- Surveyed 1,200,000 SNPs of high quality, based on coverage
- 90 K SNPs in the final array
- Includes SNPs associated with developmental deformities obtained from transcriptomic data
- Includes genetic sex determining region
- Includes possible homologous SNPs detected in Seriola dorsalis

## Part II: Biological validation of the array

- **1.** Population survey
- 2. Sex determination
- 3. Mating system
- 4. GtSeq development
- 5. Genetic improvement

### **1. Population Structure**

Baja Encenada 🖸

Broodstock (Caldera) <sup>〇</sup>

Caldera Punta Choros Guanaqueros

z.,

Freemantle (stock)



#### **Population Structure**



Drift Parameter

#### 2. Validation of the SNPs involved in sex determination



- Samples from Chile, USA and Australia
- Genome-Wide Association Study for sex determination

## 3. Mating system

- Communal spawning
- Asymmetric genetic contributions.



- Low number of spawners within each reproductive "season".
- Parental representation in broodstock collection

#### **GENETIC CONTRIBUTIONS**



#### Inbreeding measures in broodstock



F<sub>is</sub>

#### SNP parentage panels development using GTseq

Minimum allele frequency across different populations.

Mendelian Segregation

allele frequency across different populations.

#### MAF of SNPs in the validation panel





### 4. Genetic improvement

• Scope for genetic gain

Heritability of harvest weight RAS

Model	<b>h</b> <sup>2</sup>	SD <i>h</i> <sup>2</sup>
days at harvest + tank	0.596	0.048
days at harvest + tank + sex	0.590	0.048

#### Test of best strategies for genetic improvement



### Test of best strategies for genetic improvement



### Conclusions

- Investment in genomic resources is require for a correct understanding of the biology of Marine species.
- Sex determination in Seriola species is likely explained by a single gene, but further analysis is required to understand the causality of these findings.
- Development of sustainable aquaculture industries using wild species, require an in depth knowledge on genomics, to help bringing sound breeding programs.

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