Mitochondrial DNA analysis as a tool for family and species identification of fish larvae: *Emphasis on Snappers.*

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Family: Lutjanidae (snappers)

- Commercially important
- Mainly tropical and subtropical marine
- 17 genera 103 species
  - 4 Subfamilies:
    1) Lutjaninae
    2) Etelinae
    3) Apsilinae
    4) Paradicichthyinae

Subfamilies:

1. **Lutjaninae**
   - » *Lutjanus sp.* (10)
   - » *Ocyurus chrysurus*
   - » *Rhomboplites aurorubens*
2. **Etelinae:**
   - » *Etelis oculatus*
   - » *Pristipomoides macrophtalmus*
   - » *Pristipomoides aquilonaris*
3. **Apsilinae**
   - » *Apsilus dentatus*

Lutjanus jocu **(Dog snapper: Pargo perro o jocú)**

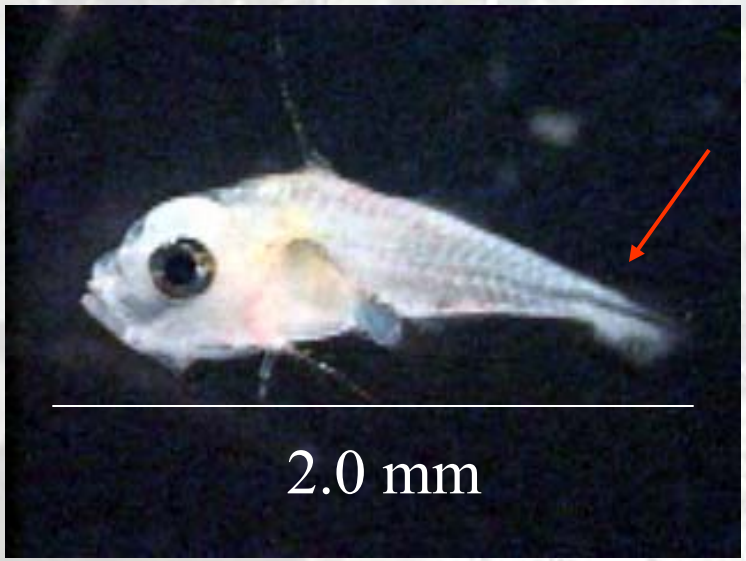
Lutjanus vivanus
(Silk snapper: Chillo "ojo amarillo")

Etelis oculatus
Queen snapper : "Cartucho"

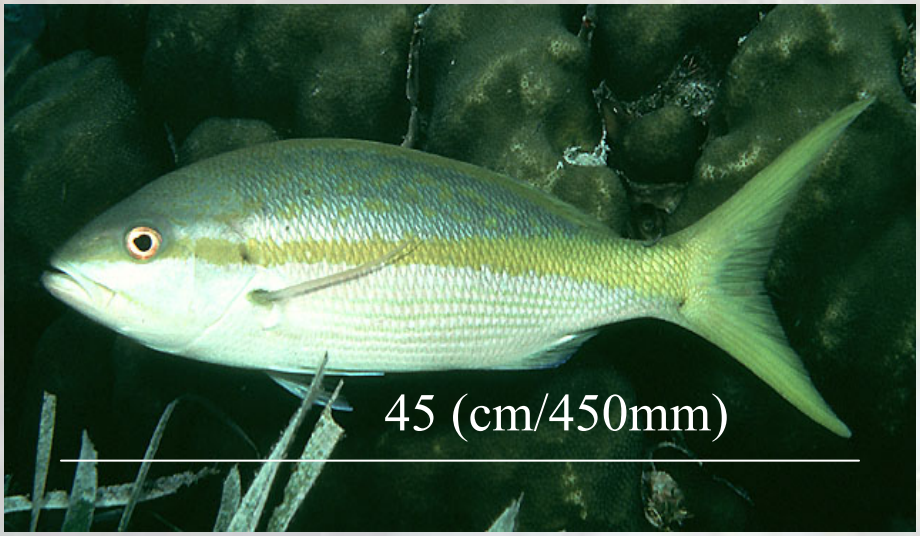
Apsilus dentatus
(Black snapper: "Chopa negra)

Problem

- **Identification of snapper larvae to the species level is crucial for the understanding of their life cycle.**
- **Show very similar larvae but very different from adults.**
- **Existing identification keys based on morphometry are useless for most larvae (pre-flexion).**

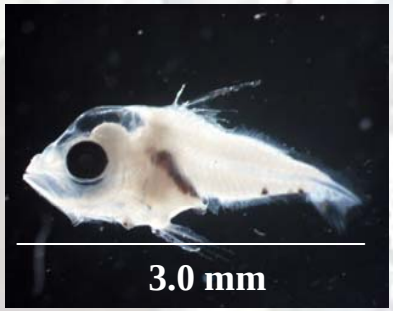


Pre - flexion larvae

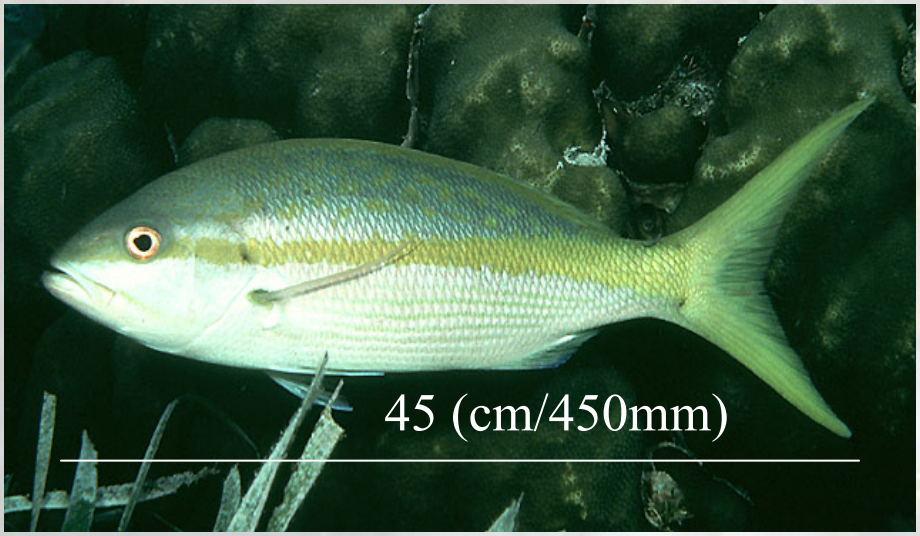


Adult – Yellowtail snapper

Larvae – Unknown species



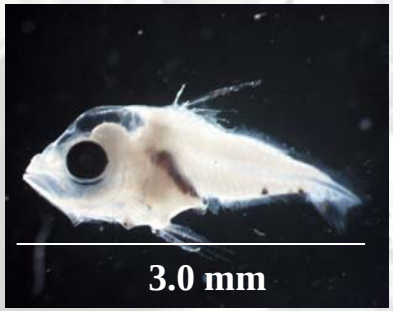
Larvae – Unknown species



Adult – Yellowtail snapper

Larvae – Unknown species

~ 300X

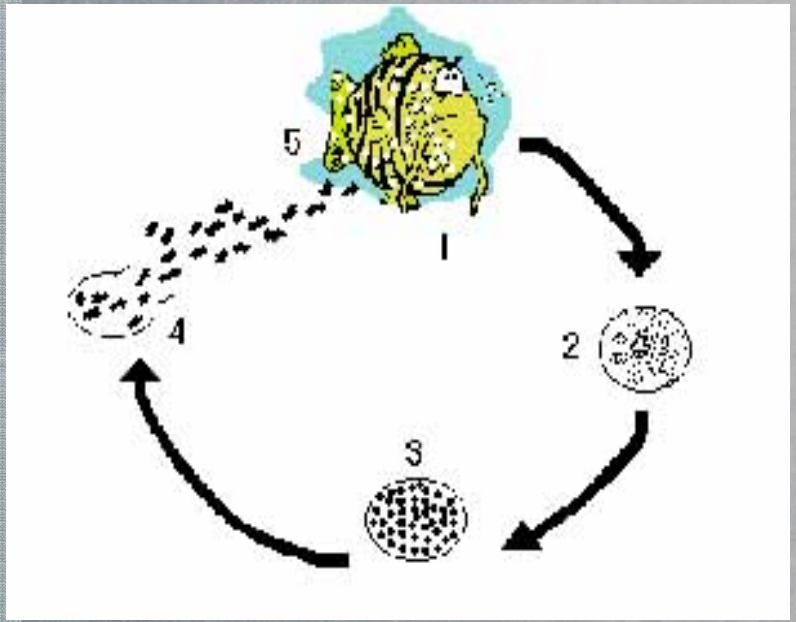


Larvae – Unknown species

Project Relevance

- **Taxonomic identification of snapper larvae to the species level represents one of the main bottlenecks in our understanding of their early life cycle and dispersal patterns.**

- **Important to evaluate management options to restore the declining stocks of commercially exploited coral reef fishes, as for marine protected areas (MPAs).**



Main Objective

Identify snapper larvae [to species] by analysis of their mtDNA 12S rRNA gene sequence; comparing them to analog adult sequences

Methods

Collection

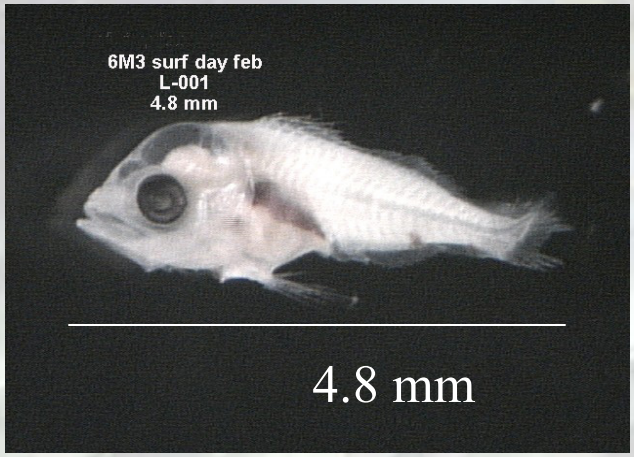
- Adults – Tissue from local catches
- Larvae – Plankton
  - Sampling sites: at La Parguera shelf edge
    - “El Hoyo”
    - “La cuarta mella”
  - Preserved in 3% ethanol

- Standard measurements and photo documentation for each larvae

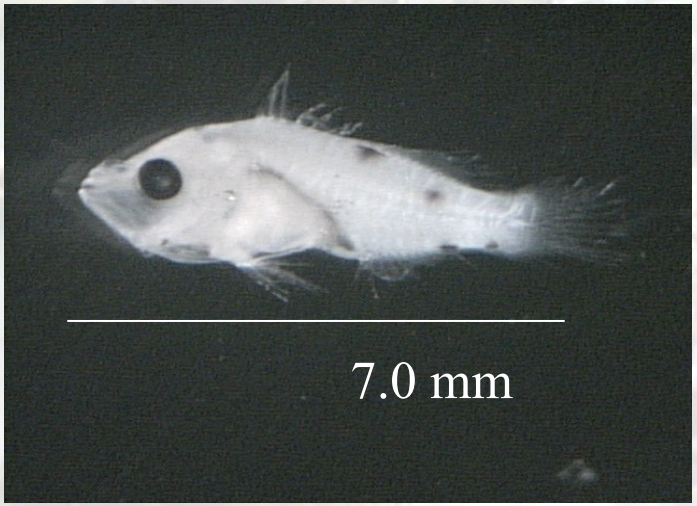
Methods

- **DNA Extraction (Qiagen Mini kit)**
  - **Adults: muscle tissue**
  - **Individual larvae**
- **PCR: mtDNA 12S rRNA gene ~ 450 bp**
- **Restriction fragment leght polymorphisms (RFLP's) : 11 enzymes**
- **Sequencing**
- **Sequence analysis: (MEGA 3.1)**
  - **Alignment**
  - **Tree construction**

Results



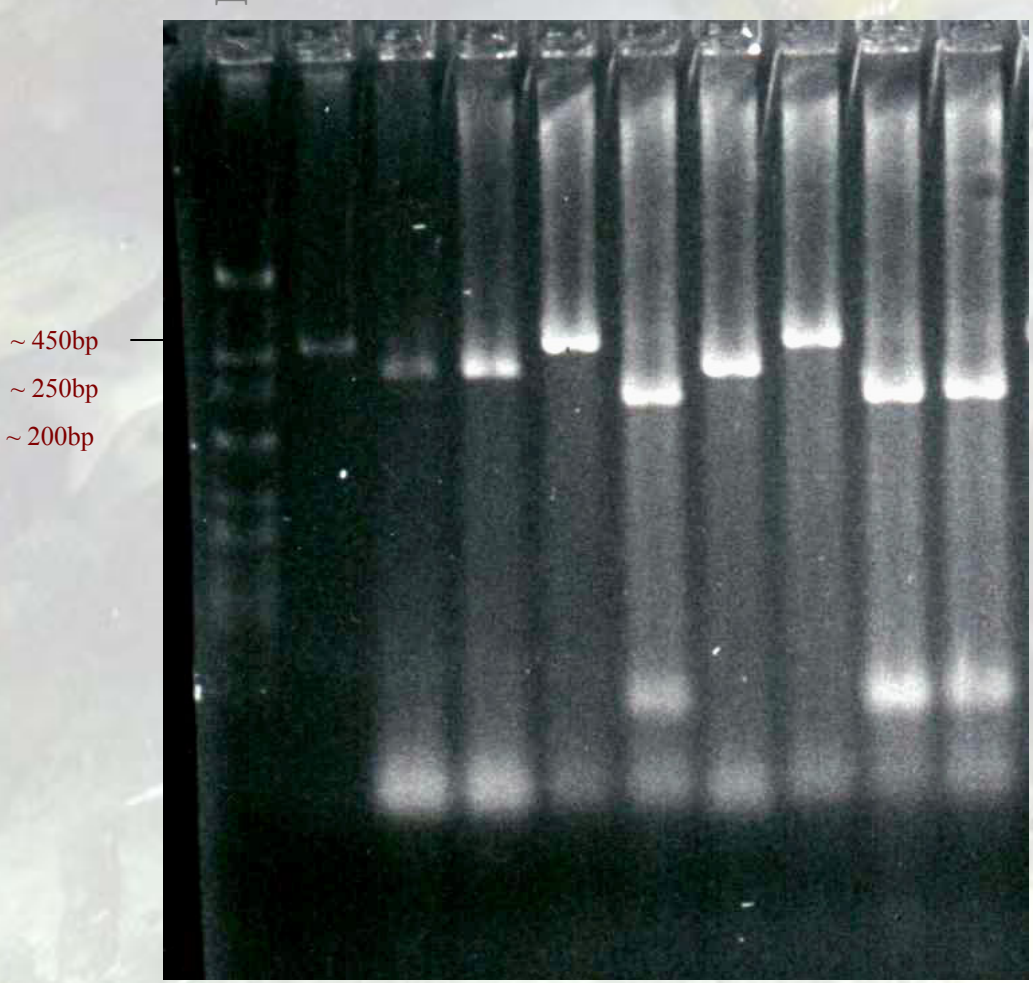
Type 1 larvae



Type 2 larvae

Two types of larvae morphologically characterized

RFLP's

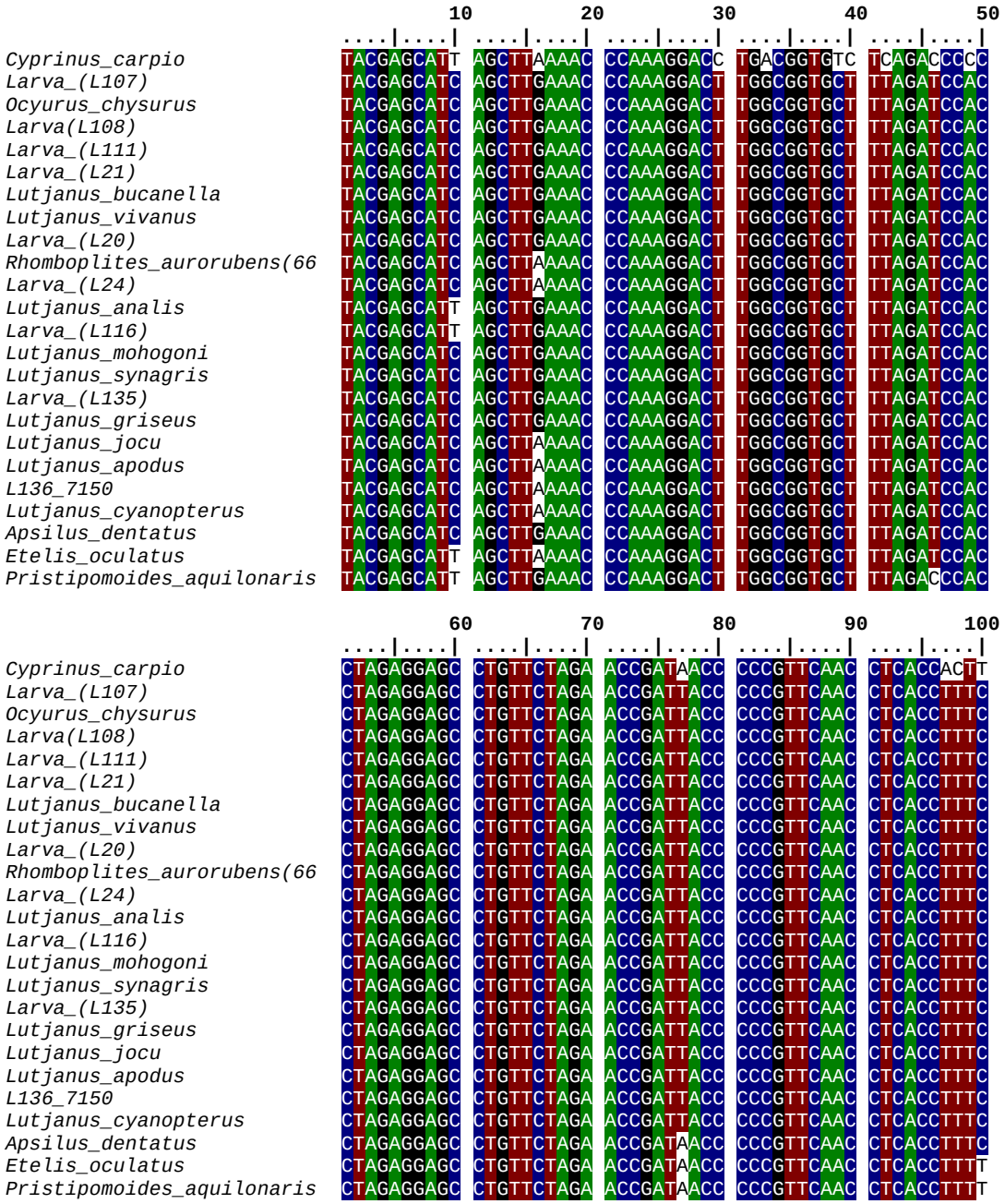


Restriction analysis with *Mse* I showing digestion patterns 0, 1 and 2.

RFLP's

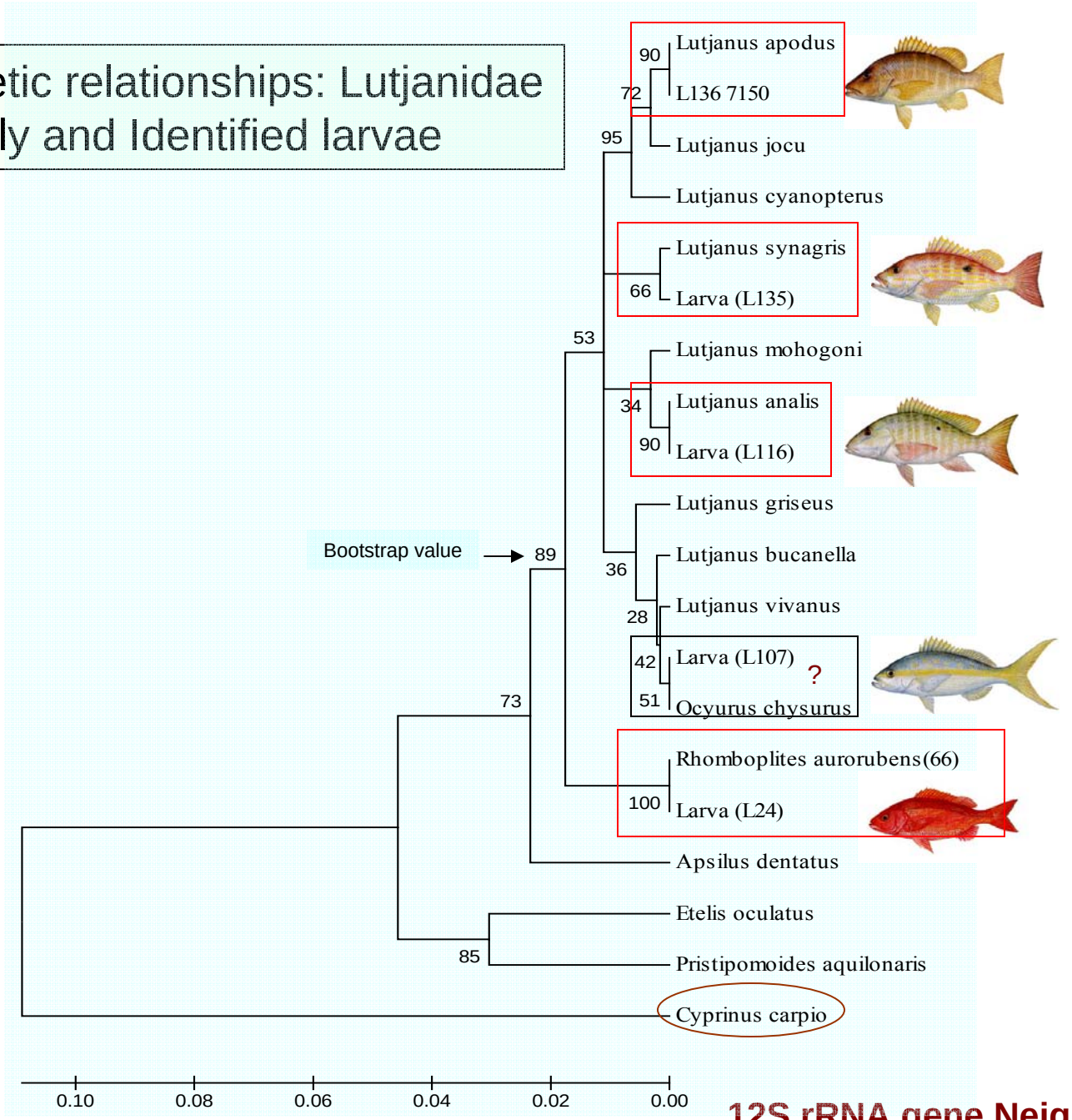
		Enzymes		
Species	*Alu* I	*Hinf* I	*Mse* I	**Haplotype**
Ocyurus chrysurus	2	1	1	A
Lutjanus vivanus	1	1	0	B
Lutjanus cyanopterus	2	0	2	
Lutjanus jocu	2	0	2	C
Lutjanus apodus	2	0	2	
Lutjanus griseus	1	0	0	D
Unknown larvae # 1	1	0	0	
Lutjanus synagris	2	0	0	E
Lutjanus analis	1	1	1	F
Lutjanus bucanella	1	1	1	
Unknown larvae # 2	1	0	2	G

- Seven haplotypes (8 ind. per haplotype for most cases)
- Four found in only one species and one larva



12S rRNA gene sequence alignment

Phylogenetic relationships: Lutjanidae Family and Identified larvae



12S rRNA gene Neighbor - Joining

Adult snapper (*Ocyurus chysurus*)

Type 1 larvae (Lutjanids)

Type 2 larvae identified
as *Serranus baldwini* and *Seranus tigrinus*

Serranus baldwini (adult)

Serranus tigrinus (adult)

Species Identified

Lutjanus apodus **(Schoolmaster)**

Rhomboplites aurorubens **(Vermillion snapper)**

Lutjanus synagris **(Lane Snapper)**

Lutjanus analis **(Mutton snapper)**

¿Ocyurus chrysurus? **(Yellowtail)**

Serranus baldwini **(Lantern bass)**
Serranus trigrinus **(Harlequin bass)**

Conclusion

- **Mitochondrial DNA analysis of the 12S rRNA gene is a reliable tool for family and species identification of fish larvae, as well as for phylogenetic reconstruction.**

- **This procedure is producing results that suggest that most larvae will be identified to the species level.**

Benefits

- **Information will be highly valuable to design more detailed research leading to characterize; dispersal, spawning and habitat selection for most of the species for the Lutjanidae family, that might be not accurately reported for the area.**

- **Knowledge gathered with this work leads to further research to characterize population structure and level of genetic connectivity of coral reef fishes populations within the Caribbean.**

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