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The *Astyanax scabripinnis* SPECIES COMPLEX (Teleostei : CHARACIDAE) – A model organism for evolutionary studies

Jonathan Pena Castro

Introduction

- Fish : variety of habitats, over 32,000 described species (Froese and Pauly , 2014);
- Brazil : greater richness and diversity (4,500 species; 71 families (Reis et al , 2003);
- Different sexuality strategies: sex determination varied mechanisms
- Determination→ short development time; Differentiation→ throughout life (environmental factors, endocrine and ploidy).

Introduction

- Sexual steroids: Great influence in differentiation:
  - Aromatase enzyme: androgen conversion to estrogens; growing of oocytes .

- Environmental and social factors : influence on sequential hermaphrodites .

- Heterogeneous mechanism of sex determination : (♀XX , ♂XY ; ♀ZW , ♂ZZ ; multiple sex chromosomes; multiple gene loci ; epigenetic factors , among others) .

Introduction

- *Astyanax: Dominant genera, approx. 156 spp (Eschemeyer , 2014)*
  - *Systematic poorly-defined*
  - *Morphological and molecular diversity*

- *Species complex (A. scabripinnis; A. altiparana; A. fasciatus).*

- *A. scabripinnis : 2n = 46 to 2n = 50 ; B chromosomes*

Astyanax aff. *scabripinnis*

- Small fish inhabit tributaries of altitude.
- Ovuliparous , external fertilization and no parental care → R strategists
- Sexual dimorphism (most pop.)

Details and experimental research

- *Astyanax genera,* (*A. scabripinnis)*
  - *Absence of morphologically differentiated sex chromosomes;*
  - *Skewed sex ratio in many pop. (female);*
  - *Significant rate B chromosomes in females;*
  - *Intersexuality*

Details and experimental research

- Labels: important for population studies;
- Proteins (electrophoretic migration);
- DNA and microsatellite DNA RNA, PCR, DNA sequencing, DNA barcoding (COI - Cytochrome c oxidase 1);
- Cytogenetic analyzes;
- morphological markings

Details and experimental research

- Morphological variation;
- Traditional morphometry: linear distances, angles, etc. multivariate statistics (eg. PCA, CVA, DFA)
- Geometric morphometry: "morphometric synthesis" multivariate statistics, multivariate biometrics, non Euclidean geometry and computer graphics.

Research interests

- Morphometric difference between populations;
- Degree of reproductive isolation;
- Compare karyotypic characteristics;
- Use different methodologies to build an evolutionary scenario of pop.

Research interests

- Interpretation of evolutionary patterns and differentiation ≠ pop.

Main methods & materials

- Cytogenetics: Mitotic Chromosomes (Bertollo et al., 1978);

- Molecular Citogenetics: FISH probes rDNA 5S, 18S (Pinkel et al., 1986).

Main methods & materials

- Geometric morphometry: Photos (180 dpi) standard; Anatomical landmarks;
- Analyzes information : influence of crom. B
- Sex ratio and frequency: population analysis
- Microdissection, DOP-PCR amplification.
- Template for amplification primers sex - specific;
- Product sequencing and analysis.

Main methods & materials

- Procrustes superimposition of least squares;
- Canonical variate analysis (CVA);
- Discriminant function analysis (DFA).
- Reproduction assays.

Main methods & materials

- Scanning electron microscopy (SEM) secundary sexual dimorphism

Analysis methods

- Literature Groups Confrontation (eg. *A. mexicanus*).
- DNA markers qPCR validation (methodology ΔΔct) compared to normalizing genes.
- Statistical analysis with a significance of 95% of quantitative data.
- Sequences: Comparison in genomic databases (NCBI - National Center for Biotechnology Information)
- Specific software (Ex. Geneious [Biomatters Limited])
- similarities
- Construction of primers

Analysis methods

- Procedures derived from hybridizations: Qualitative analyzes by the presence of signals

- Secondary sexual characters, reproductive period;

Obtained data

- Differentiate populations morphometric
  - Separate populations, intra- and inter dimorphism.
- Degree of reproductive isolation
  - Isolation pre-zygotic
- Compare karyotypic characteristics;
  - Differences and similarities
- Use different methodologies to build an evolutionary scenario of pop.
  - Analyzes show pop. cryptic species are distinct, independently evolving;
- Specific analyzes show differences in adaptation of fish in relation to the environment;

Obtained data

- Incipient speciation;
- Reproductive isolation;
- Absence gene flow.

Prospects

- Further knowledge B chromosome influence on populations
- Details on reproductive characteristics
- Next-generation sequencing

Prospects

- Studies for the creation and management aimed for aquaculture.

- Preservation of natural populations.

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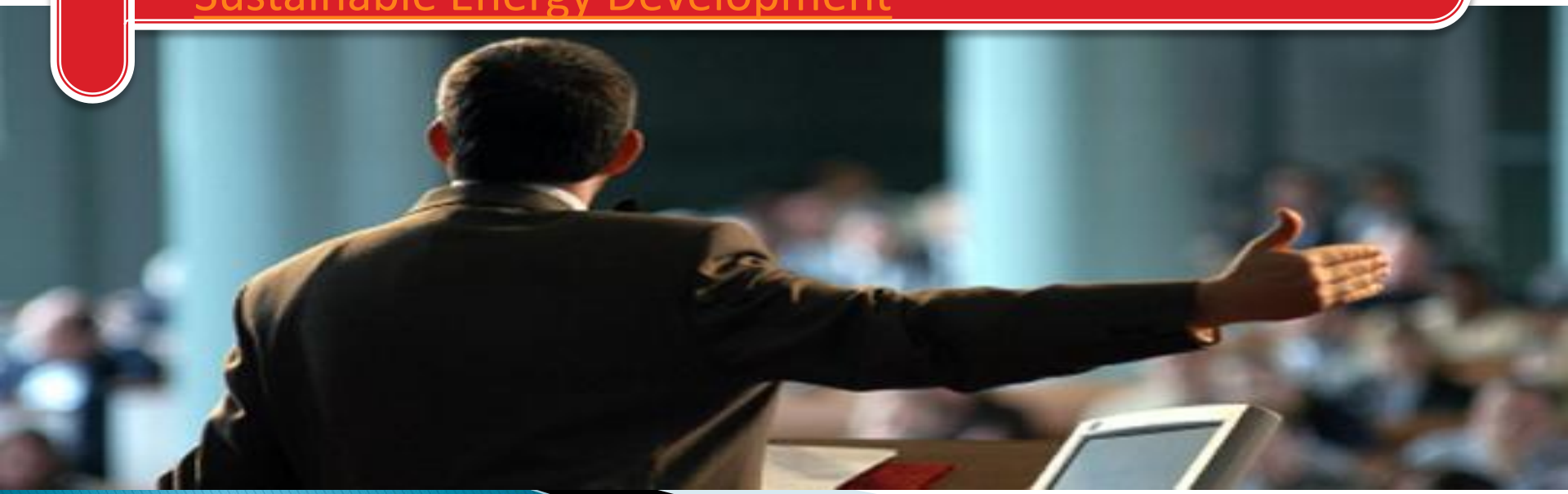

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