

DNA Barcode of Midas Cichlidae species complex inhabiting lakes and lagoons of Nicaragua.

Presented by
Lucia Paiz- Medina

Third International Barcode of Life Conference
7th -13th November 2009
Mexico DF

Nicaragua's location



Biodiversity: Animals



Class	Species
Invertebrates	263,716
Vertebrates	1,776
Mammals	183
Birds	705
Reptiles	177
Amphibious	71
Fishes	640



(Rueda, 2007)

Biodiversity: Plants

- Vascular plants (herbarium samples): 5,796
- Includes 1,699 genus of:
 - Bracken
 - Gymnosperm
 - Angiosperm
- 73 are endemic plants.



Midas Cichlidae Complex

In Nicaragua inhabit a group of fish belonging to the Cichlidae family, *Amphilophus* genus, that since the first attempt of their taxonomic classification, presented problems because of the similarity between morphological species complex.

(Mckaye and Stauffer, 2002)



M. Geiger

Midas Cichlidae Complex

Initially, because it was impossible to differentiate and classify them, it was thought that there was only one polymorphic species and, after conducting several studies, now, we know that they are different.

Amphilophus xiloaensis



Amphilophus amarillo



Midas Cichlidae Complex

The Midas Cichlidae Complex, as we called these species, is very important to science because of his evolutionary meaning.

It arises two main questions: **How many species are they?** and **Which was the process of speciation involved?**

Midas Cichlidae Complex

Midas Cichlidae Complex is comprised of species belonging to *Amphilophus* genus, that differ morphologically, and vary in feeding habits and breeding site selection.

These species inhabit small endorheic crater lakes that were formed from volcanic explosions within the past 100,000 years. (Stauffer, 2008)

Apoyeque



M.Geiger

Apoyo



From Stauffer 2002

Described species

- *Amphilophus citrinellus* (Günther, 1864)
- *Amphilophus labiatus* (Günther, 1864)
- *Amphilophus zaliosus* (Barlow, 1976) endemic of Apoyo lagoon
- *Amphilophus sagittae* Stauffer & McKaye, 2002
- *Amphilophus amarillo* Stauffer & McKaye, 2002
- *Amphilophus xiloaensis* Stauffer & McKaye, 2002
endemics of Xiloa lagoon
- *Amphilophus chancho* Stauffer, McCrary & Black, 2008
- *Amphilophus flaveolus* Stauffer, McCrary & Black, 2008
- *Amphilophus astorquii*, Stauffer, McCrary & Black, 2008
endemics of Apoyo lagoon



Amphilophus citrinellus



Sympatric Speciation

Mechanism of speciation in this complex is one of the most important question because:

- Lagoons are of recent origin
- Those lagoons are small , species had to evolved quickly in a limited area.

(Barluenga et al, 2006)

Sympatric Speciation

To infer that sympatric speciation might be occurring:

- Encounter two or more phenotypically discernible populations of monophyletic origin.
- Morphological, ecological, genetic and phylogenetics analysis have to be done.
(McKaye et al, 2002)

Sympatric Speciation

Vol 439|9 February 2006|doi:10.1038/nature04325

nature

LETTERS

Sympatric speciation in Nicaraguan crater lake cichlid fish

Marta Barluenga^{1*}, Kai N. Stölting^{1*}, Walter Salzburger^{1,2*}, Moritz Muschick¹ & Axel Meyer¹

This study includes phylogeographic , population-genetic, morphometric and ecological analyses.

Sympatric Speciation

Concludes that

- Crater lake Apoyo was seeded only once by *A. citrinellus*
- A new species, *A. zalius*, evolved in the lake from the ancestral species within less than 10,000 years
- The two species in Lake Apoyo are reproductively isolated
- The two species are eco-morphologically distinct

Even when it is very probable that sympatric speciation has occurred, several researchers think that it is necessary to study it more to confirm that.

A. citrinellus



From Barluenga et al, 2006

A. zalius



M. Geiger

DNA Barcode project

Started in 2007 with a collaboration between **Centro de Biología Molecular**, Universidad Centroamericana ; **Jeffrey McCrary** (FUNDECI-GAIA) and **Matthias Geiger** (Zoologische Staatssammlung München & Ludwig Maximilian Universität München)

DNA Barcode project

Started with the aim of promoting national investigation and to contribute to the study of the Midas Cichlidae Complex in Nicaragua.

Currently the **work-team** is conformed by:

Jorge Huete-Perez

Lucia Paiz Medina

Jeffrey McCrary

Matthias Geiger

Objectives

- Identify how many species are in the Complex using CO1
- Differentiate species inhabiting in each lagoon (Phylogenetics)
- To infer the speciation mechanism
- To establish the Barcoding methodology in Nicaragua

DNA Barcode project

Thanks to the initial collaboration, it was collected 239 fin samples



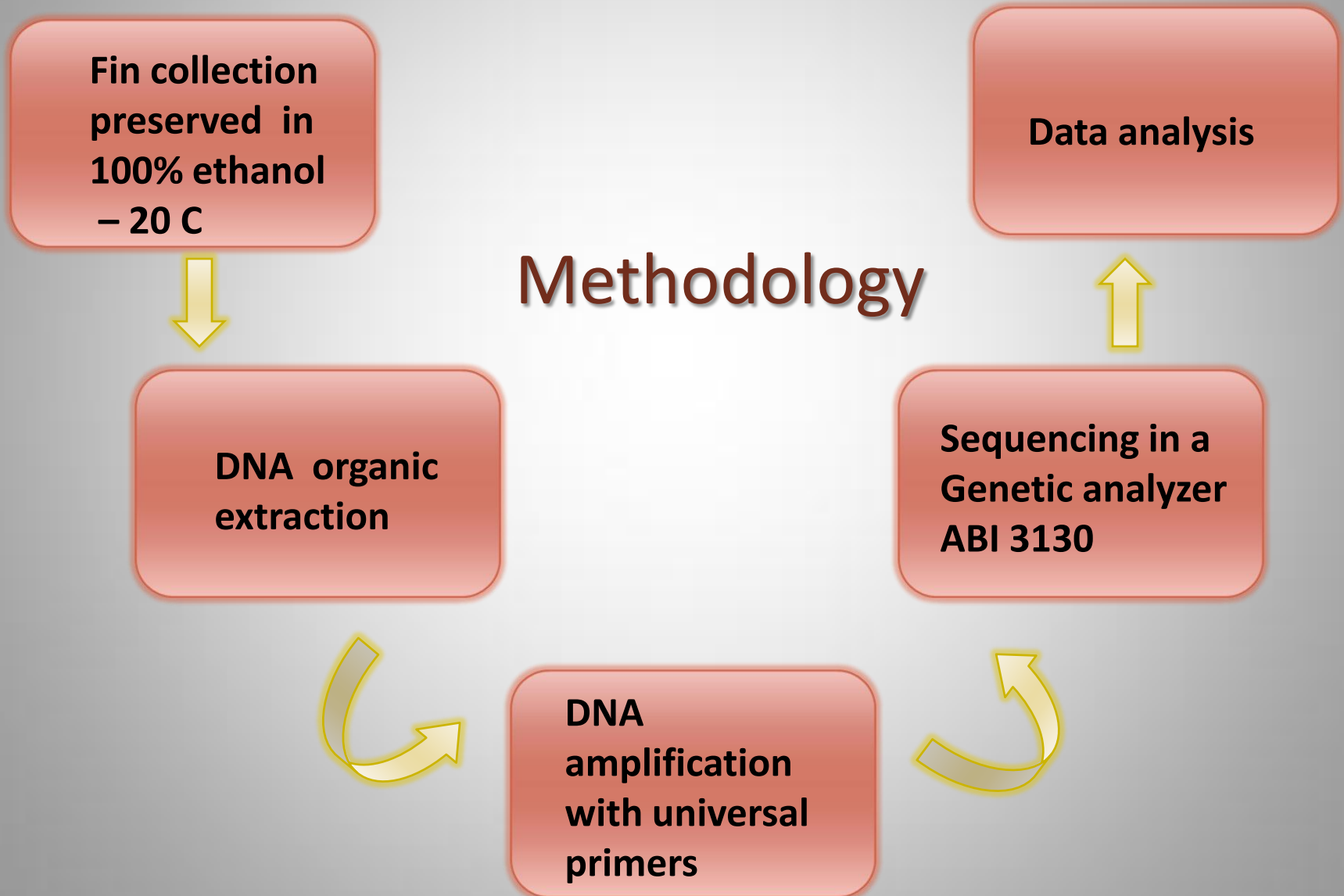
By M. Geiger

DNA Barcode project: Sampling location



From Stauffer, 2002

DNA Barcode project



Primers

16S

16Sar-5' CGC CTG TTT ATC AAA AAC AT

16Sar -3' CCG GTC TGA ACT CAG ATC ACG T

COI-1

FF2d TTC TCC ACC AAC CAC AAR GAY ATY GG

FR1d CAC CTC AGG GTG TCC GAA RAA YCA RAA

COI-2

LepF1_t1 1 TGATAAACGACGGCCAGTATTCAACCAATCATAAAGATATTGG

VF1_t1 1 T GTATAAACGACGGCCAGTTCTCAACCAACCACAAAGACATTGG

VF1d_t1 1 TGATAAACGACGGCCAGTTCTCAACCAACCACAARGAYATYGG

VF1i_t1 3 TGATAAACGACGGCCAGTTCTCAACCAACCAIAAIGAIATIGG

LepRI_t1 1 CAGGAAACAGCTATGACTAAACTTCTGGATGTCCAAAAAATCA

VR1d_t1 1 CAGGAAACAGCTATGACTAGACTTCTGGGTGGCCRAARAAYCA

VR1_t1 1 CAGGAAACAGCTATGACTAGACTTCTGGGTGGCCAAAGAATCA

VR1i_t1 3 CAGGAAACAGCTATGACTAGACTTCTGGGTGICCIAAIAAICA

COI-3

VF2_t1 1 TGATAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC

FishF2_t1 1 TGATAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC

FishR2_t1 1 CAGGAAACAGCTATGACTTTCAGGGTGACCGAAGAATCAGAA

FR1d_t1 1 CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA

M13F (-21) TGATAAACGACGGCCAGT

M13R (-27) CAGGAAACAGCTATGAC

Ivanova et al.
2007

FishF1-5' TCA ACC AAC CAC AAA GAC ATT GGC AC 3'

FishF2-5' TCG ACT AAT CAT AAA GAT ATC GGC AC 3'

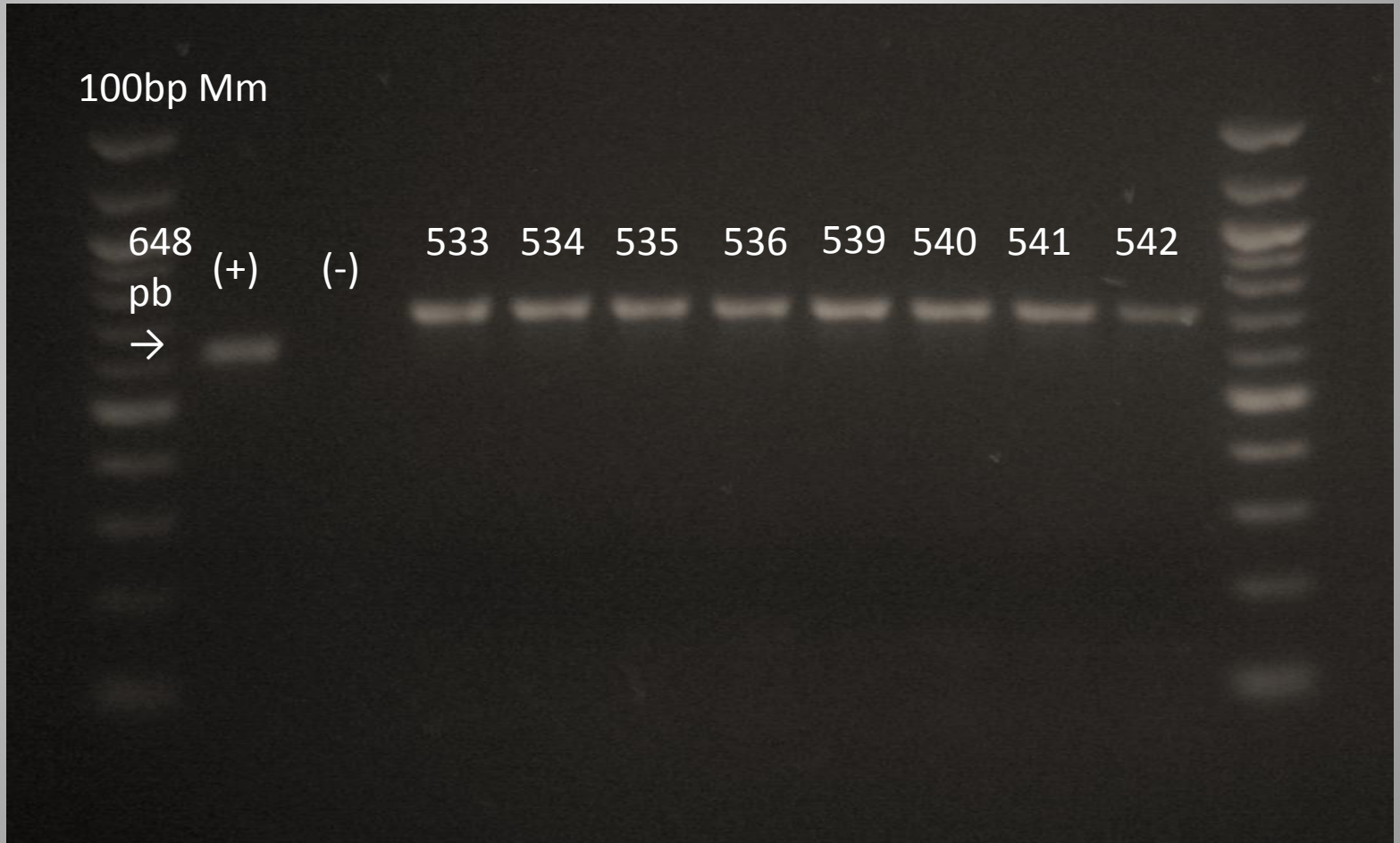
FishR1-5' TAG ACT TCT GGG TGG CCA AAG AAT CA 3'

FishR2-5' ACT TCA GGG TGA CCG AAG AAT CAG AA 3'

Ward et al.
2005

DNA Barcode project: Amplification

Fish F1-Fisf R1



DNA Barcode project: Coming activities

- Amplification of the whole DNA collection
- Sequencing
- Data analyses
- Publication



From Stauffer, 2002

Thank you for your kind attention!



Molecular Biology Center Work Team