



UNIVERSIDAD AUTÓNOMA DEL
ESTADO DE MORELOS



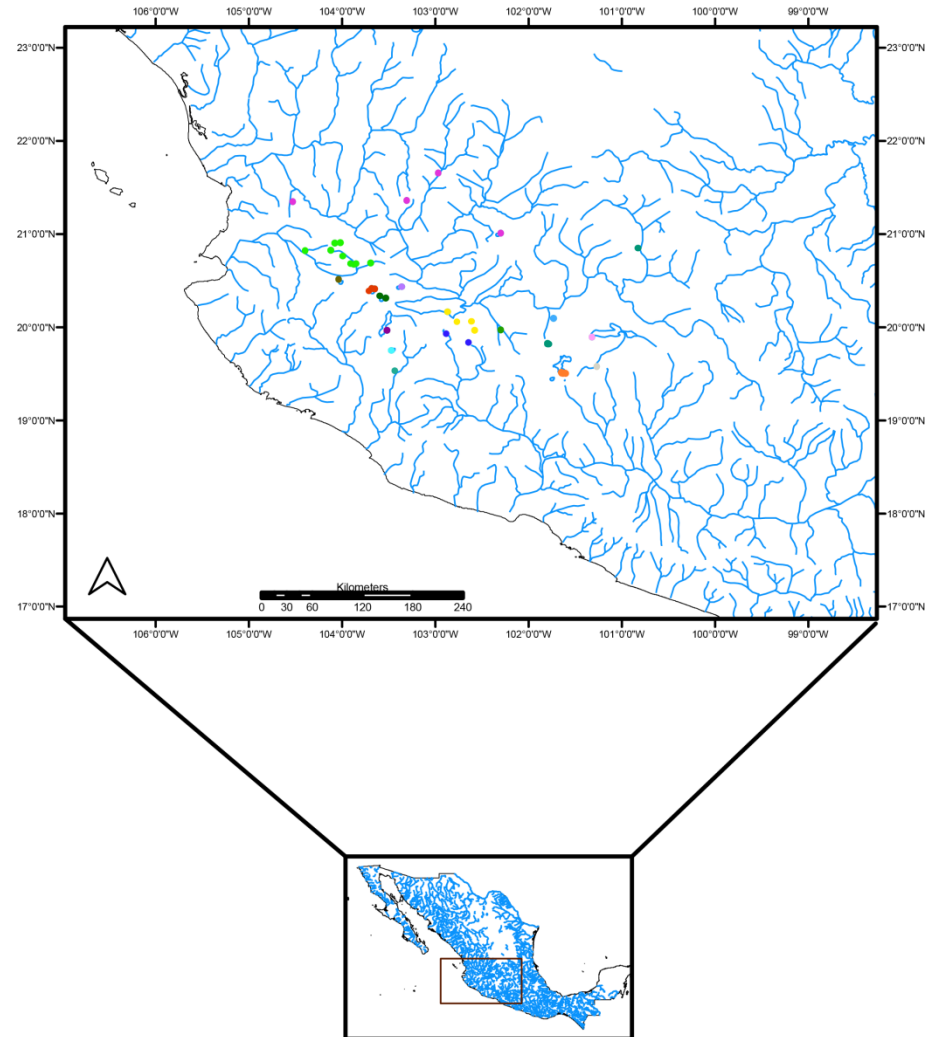
Molecular phylogeny of the species of the genus *Ilyodon* (Goodeidae)

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and Omar Domínguez Domínguez

BACKGROUND

- Volcanic and tectonic activities
- Miocene, Pliocene, Pleistocene
- continues cycles of communication and isolation over the past million years
- High richness of freshwater fishes species (near to 100 spp.)
- High number of endemism (near to 70%)

Central Mexico



(Barbour, 1973; Smith, 1980; Ferrari et al. 2000).

FAMILY GOODEIDAE

- 20 genera
- 42 species
- Subfamily Goodeinae





I. whitei (Meek, 1904)



I. furcidens (Jordan y Gilbert, 1882)



I. amecae (Radda, 1989)



I. lennoni (Meyer y Foerster, 1983)



I. xantusi (Hubbs y Turner, 1939)



I. cortesae (Paulo-Maya y Trujillo-Jimenéz, 2000)

Controversies in the number of species that comprise the genus *Ilyodon*

- **Turner y Grosse (1980)** compare 5 allozyme loci and proposed that *I. furcidens* and *I. xantusi*, are not different species, but have different trophic morphotypes
- **Doadrio and Domínguez (2004)** recognized three species: *I. whitei*, *I. furcidens* and *I. amecae* (0.01-1.7%).
- **Webb et al. (2004)** recognized two species: *I. whitei* and *I. furcidens* (0.8%).
- **Miller et al. (2005)** recognized only to *I. whitei* and *I. furcidens*
- **Domínguez-Domínguez et al. (2010)** they described to *I. whitei* and *I. furcidens* as paraphyletic species, because *I. whitei* is grouped like a polytomy unresolved that included to *I. lennoni*, *I. cortesae* and at least one population of *I. furcidens*

- The present study represent the first one in which it's include samples of all distribution range of species of genus *Ilyodon* and the first that include mitochondrial and nuclear loci.

- The aims of this work were:

1. Infer the genus evolutionary history and relationships between all recognized species
2. Establish the time and the way of diversification of main clades recovered

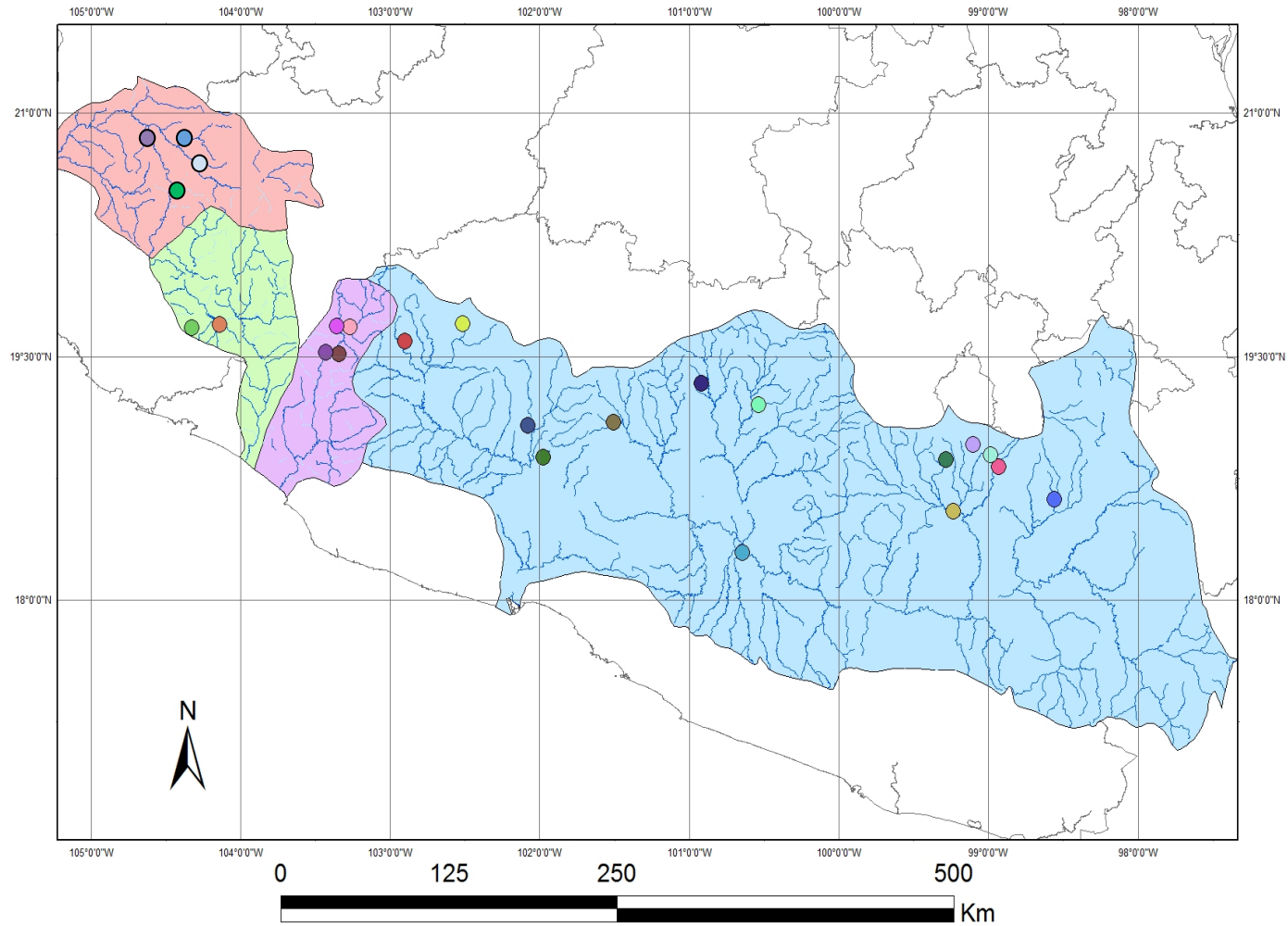
Also, considering that *Ilyodon whitei* is catalogued in the IUCN Red List of Threatened Species (IUCN, 2015)

and another specie is in the NOM-059 (SEMARNAT, 2010)

result important clear up the phylogenetic relationships of the species that composed the genus *Ilyodon*, to establish appropriate conservation programs.

METHODS

84 individuals



DNA Extraction

PCR

Edition & Alignment

Were amplified fragments of three mitochondrial genes:
Cytochrome b (*Cytb*)
Subunit I of Cytochrome Oxidase I (*COI*)
(*Dloop*) of the control region
nuclear gene B-actin (*RAG1*)
4032 pb

Partition homogeneity test in PAUP was conducted to evaluate the significance of conflict among data sets, using 1000 resampling of characters.

Model selection based on Akaike information criterion (AIC) and optimal partition setting analysis were performed using PartitionFinder v1.1.0 (Lanfear et al. 2012). We treated each gene as a single locus.

Haplotype network

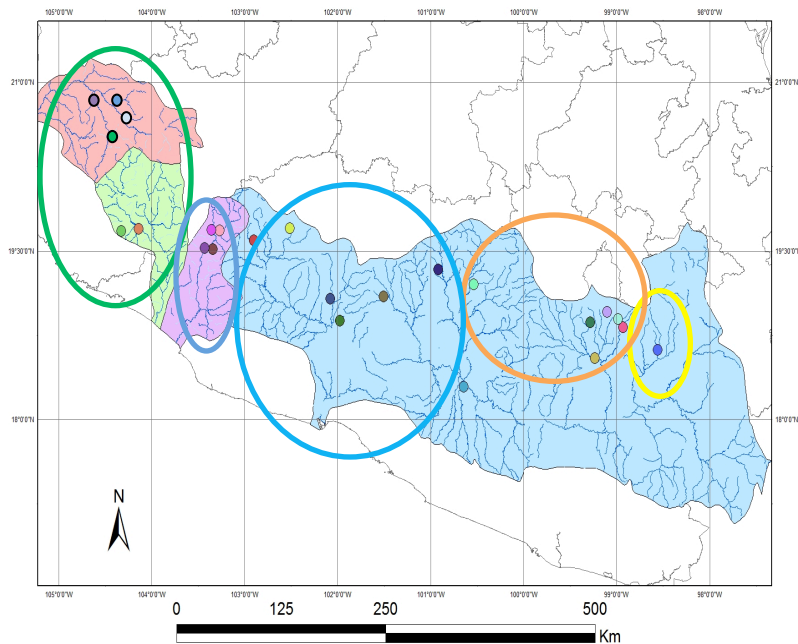
Gene trees were constructed using Bayesian inference (BI): Mr. Bayes and Maximum likelihood (RAxML)

Divergence times estimation: (Cytb) molecular clock calibrated with the mutation rate calculated for the teleosts fishes

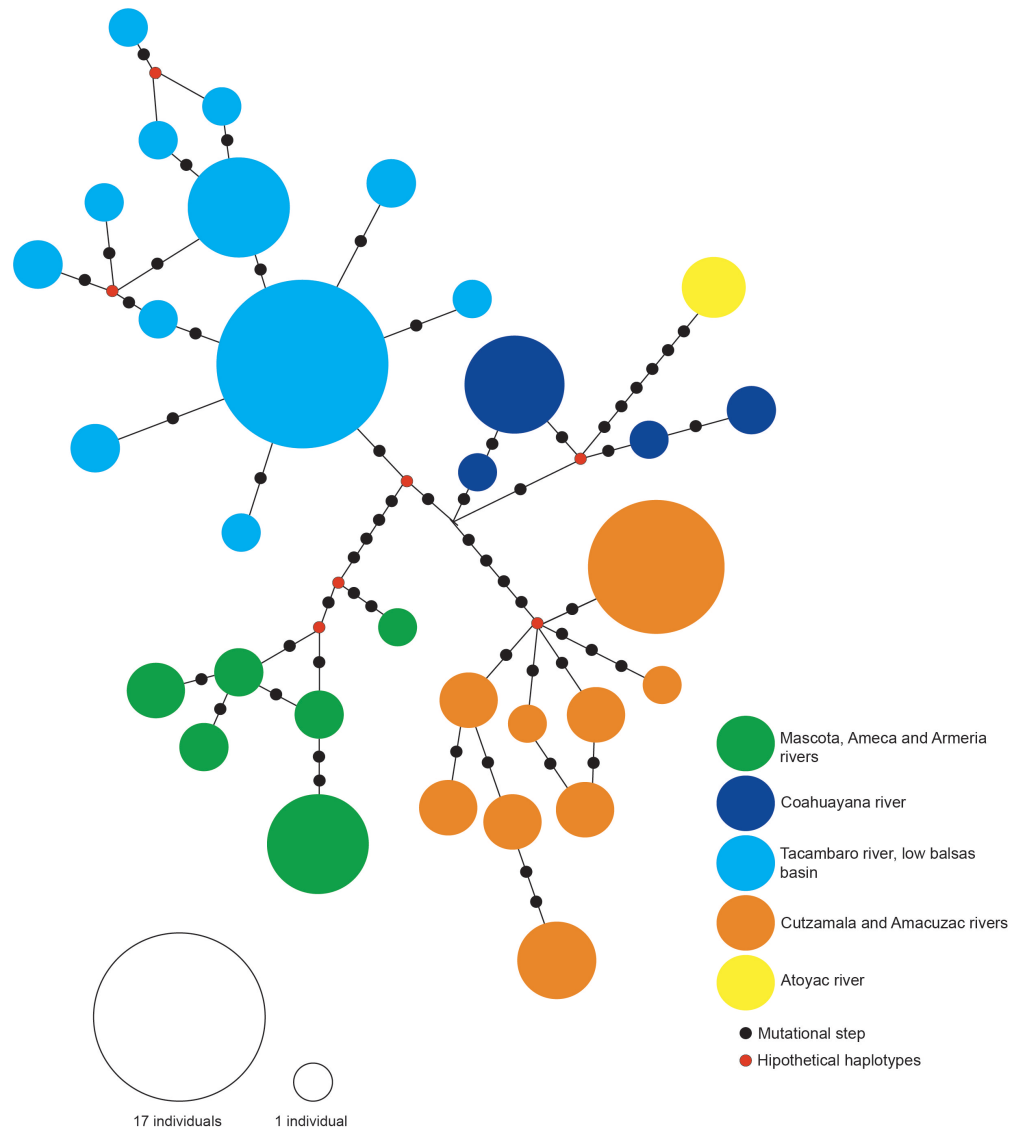
Uncorrected genetic distances “p” with Cytb and COI (Mega v5.1)

AMOVA: how is partitioned the genetic variance at various hierarchical levels
Cytb
COI

RESULTS

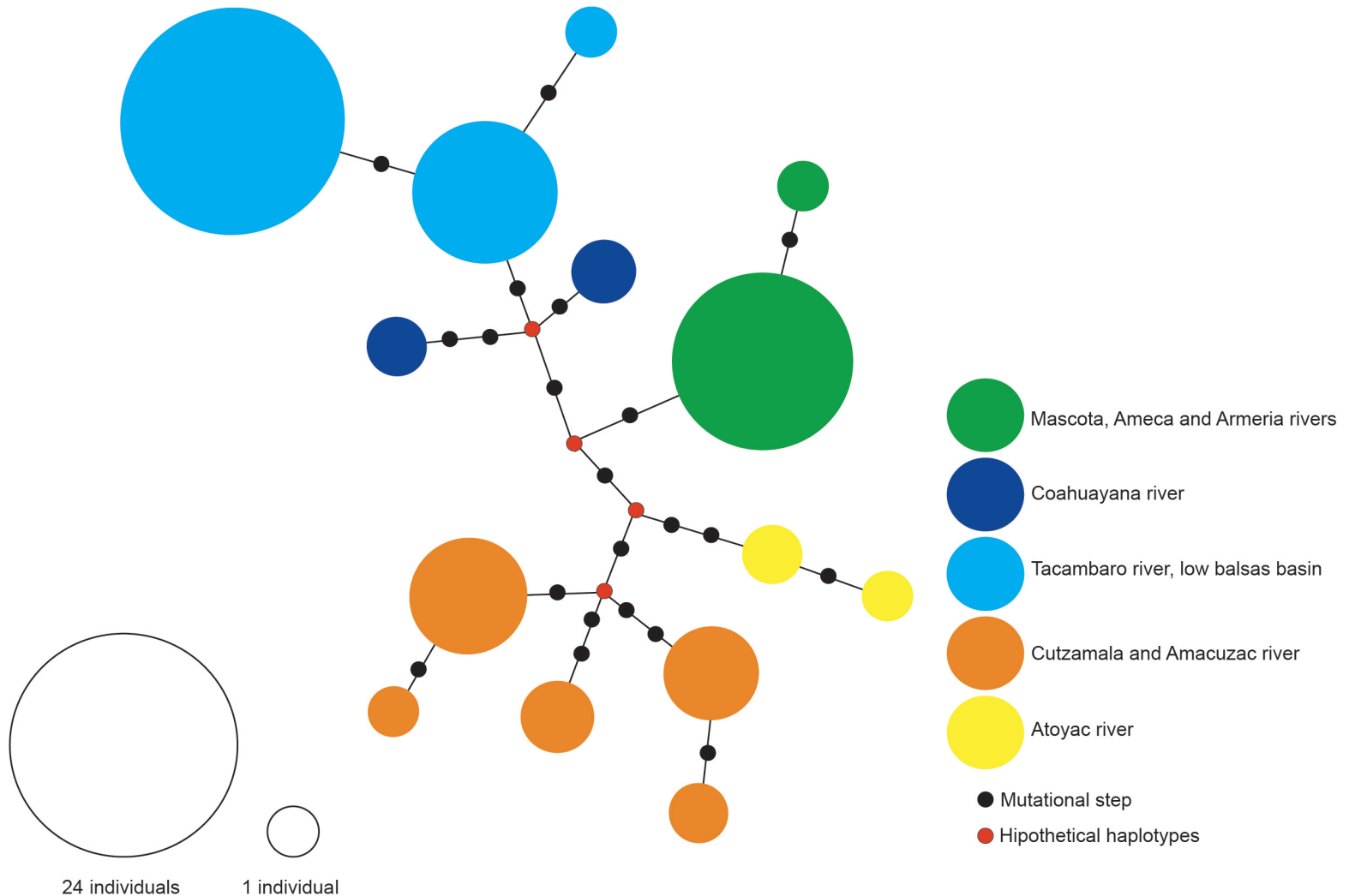


Cytb
One to 12 mutational steps



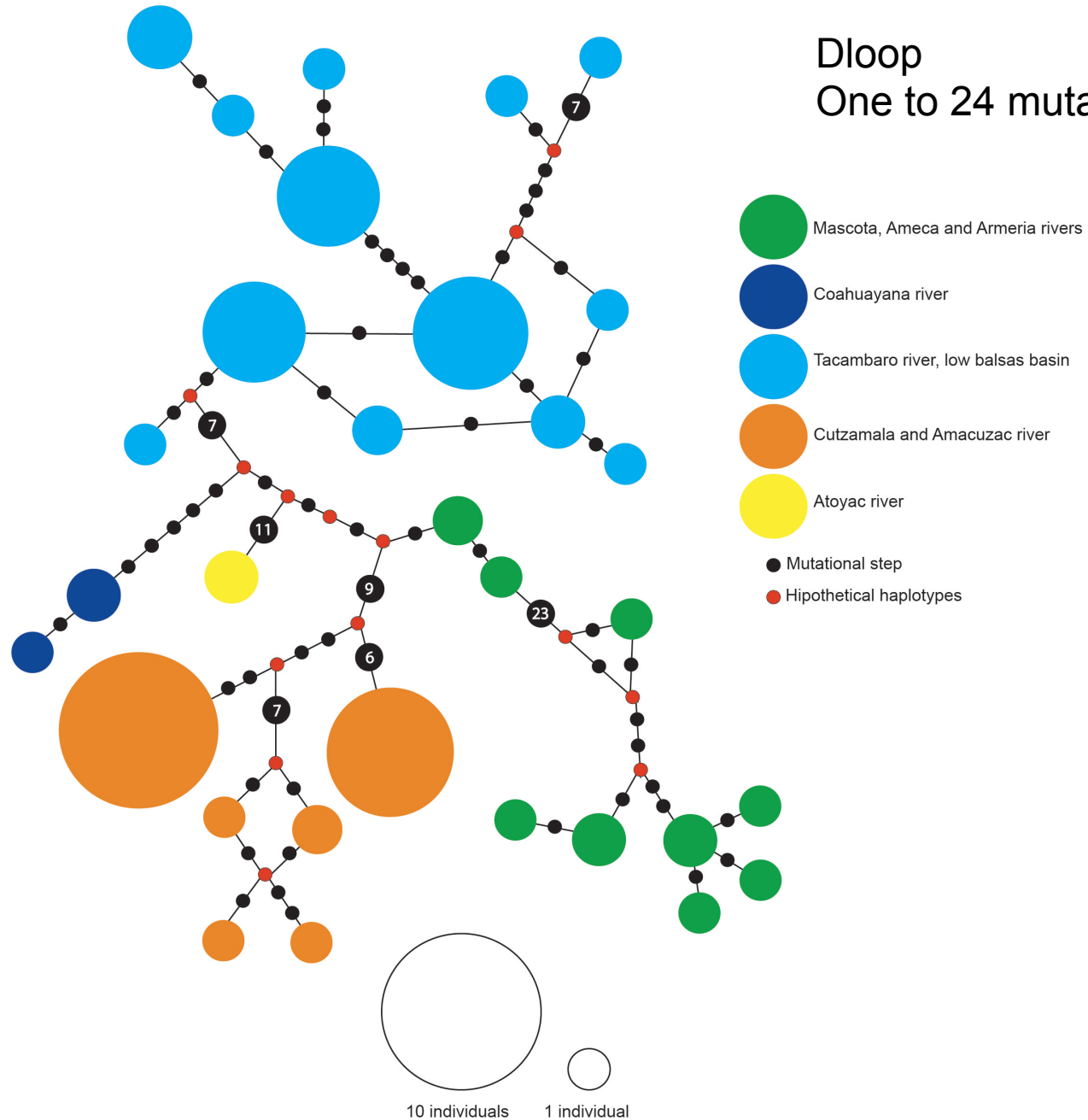
COI

One to nine mutational steps

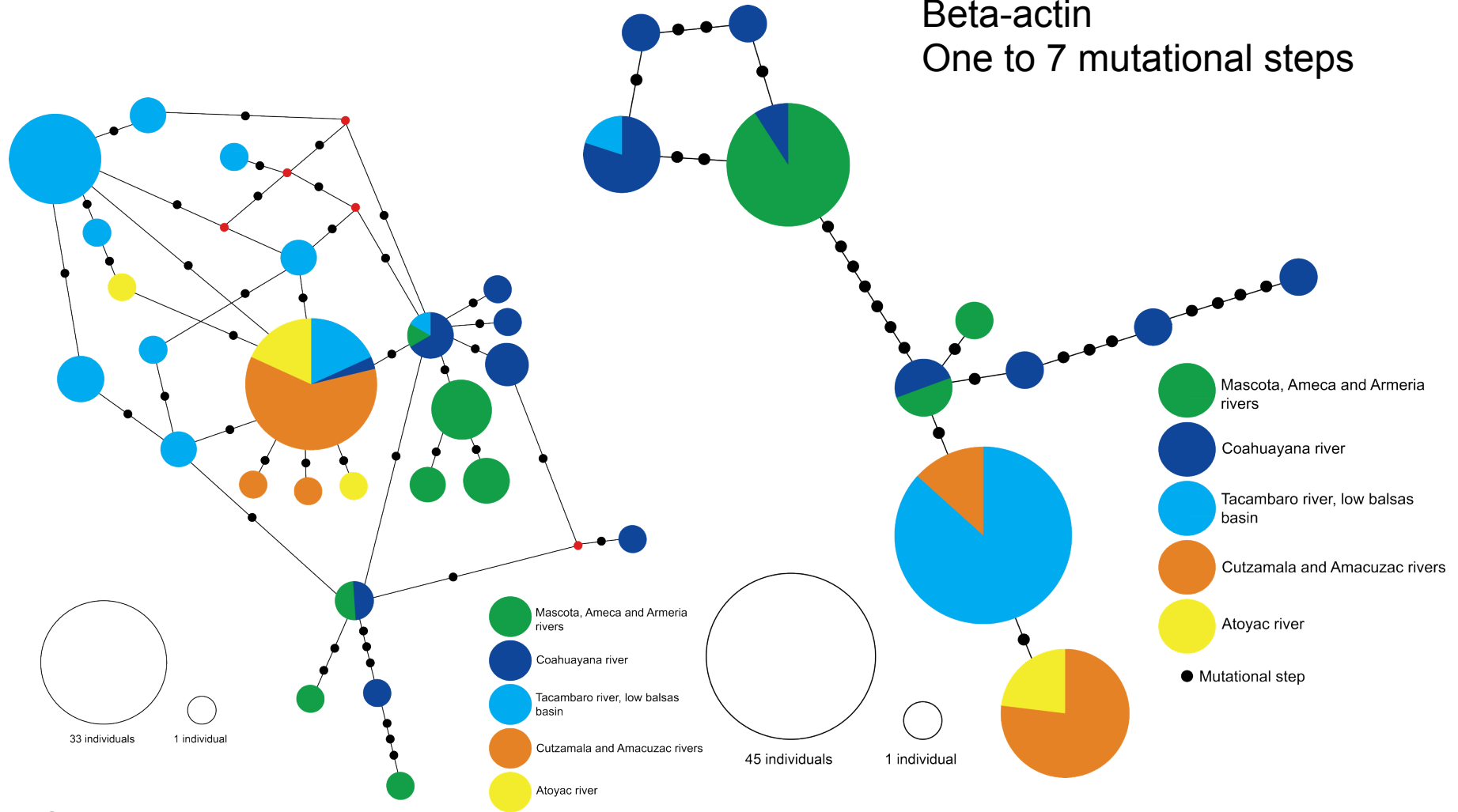


Dloop

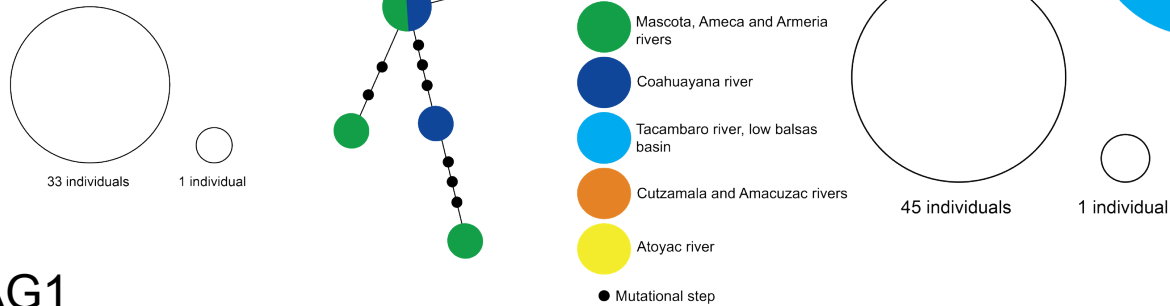
One to 24 mutational steps



Beta-actin One to 7 mutational steps

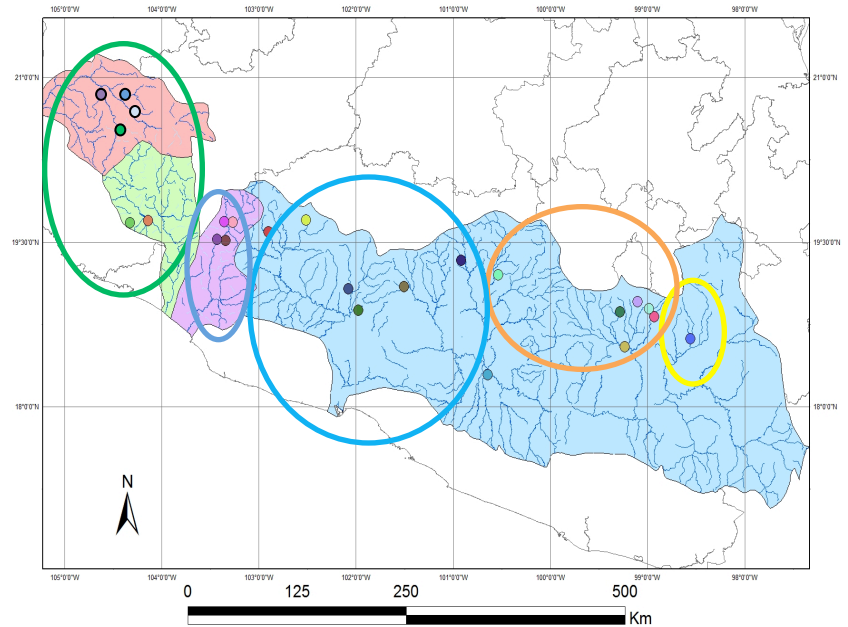
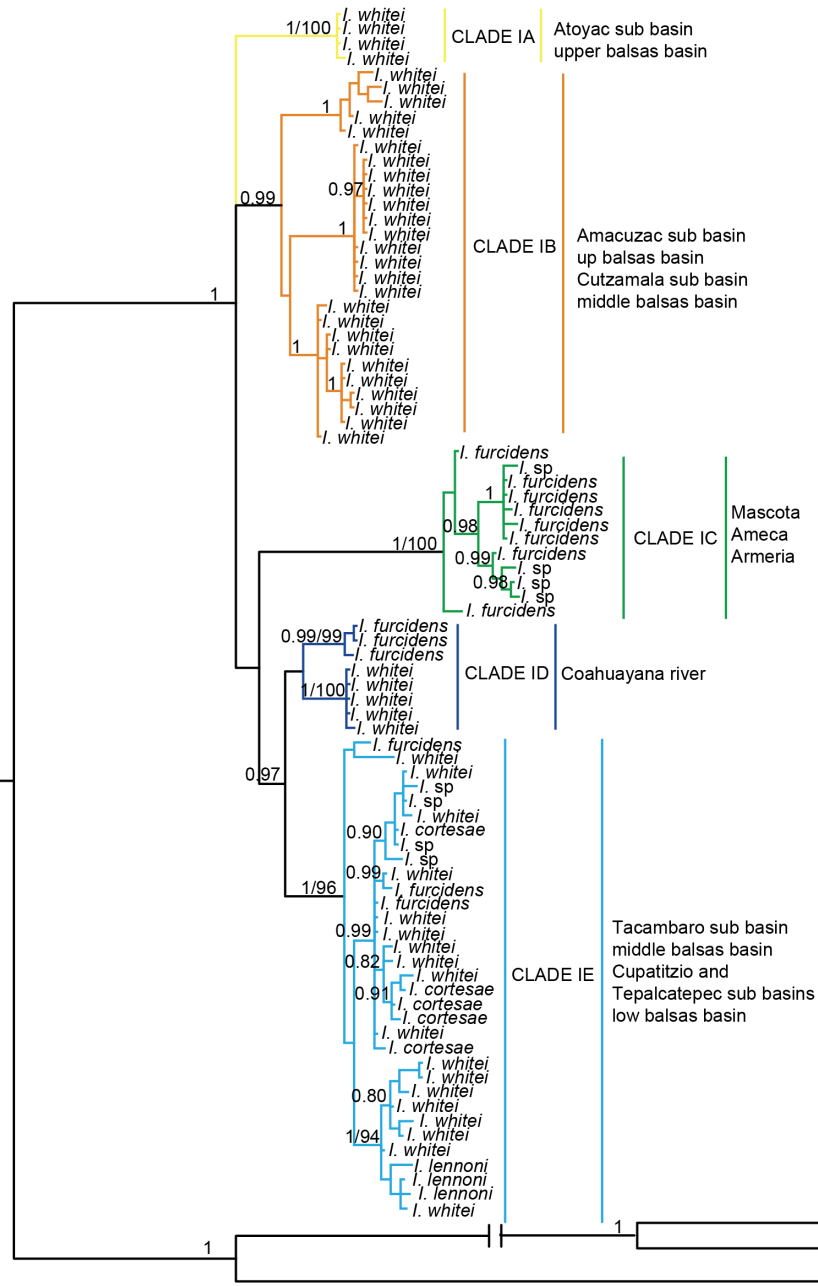


RAG1 One to 3 mutational steps

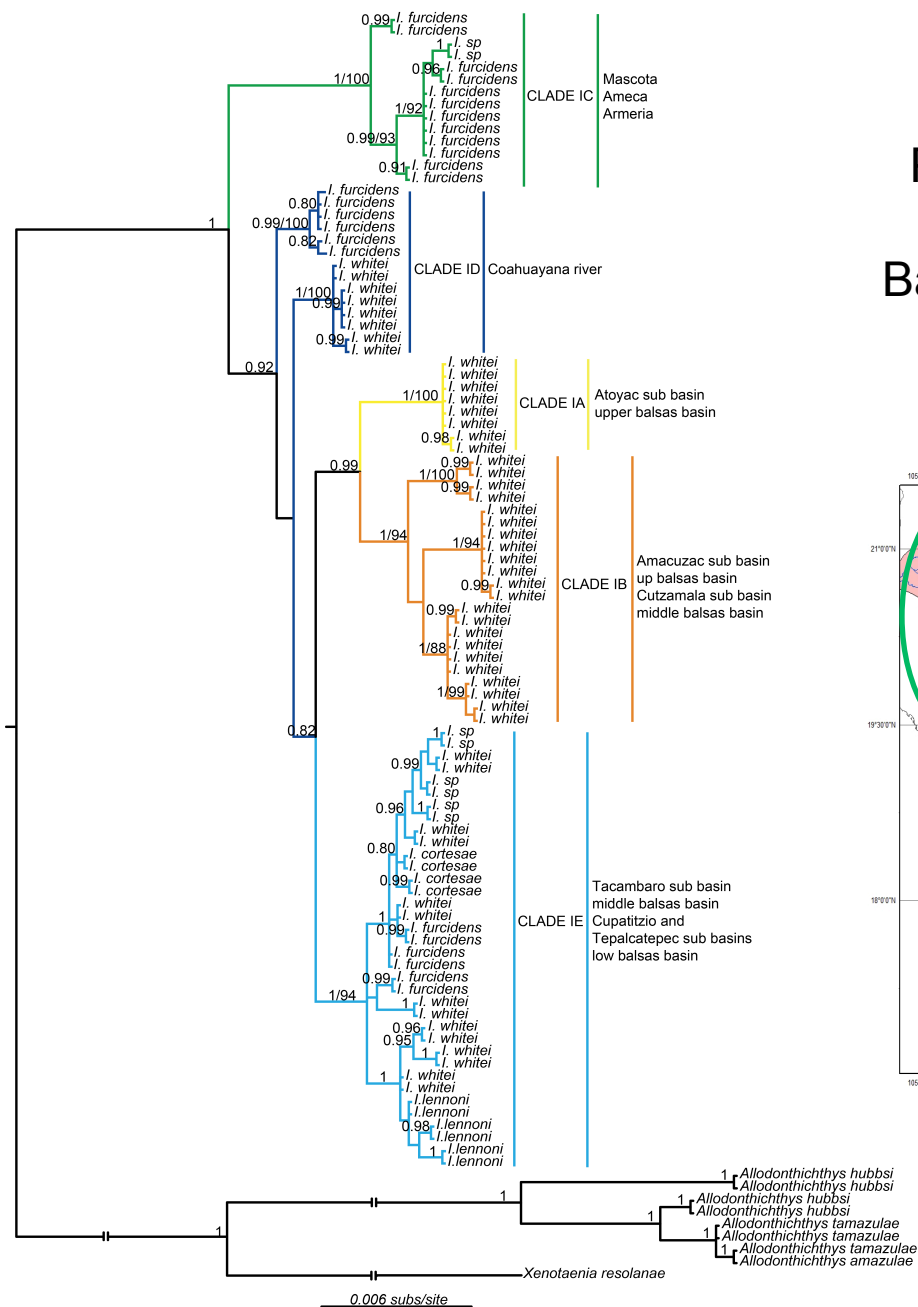


Phylogenetic tree with mtDNA

Bayesian posterior probabilities/Bootstrap probabilities

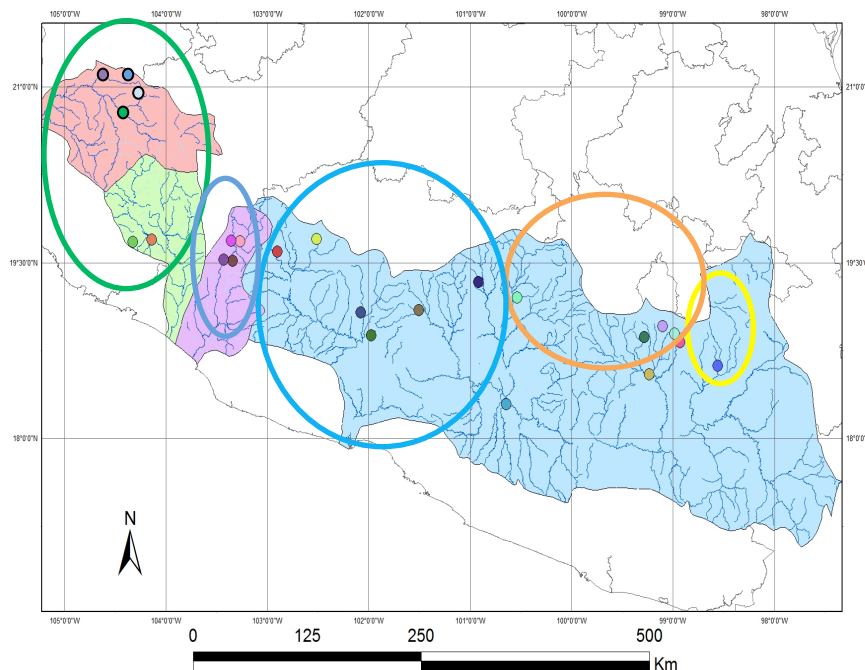


0.02 subs/site



Phylogenetic tree with mtDNA+nDNA

Bayesian posterior probabilities/Bootstrap probabilities

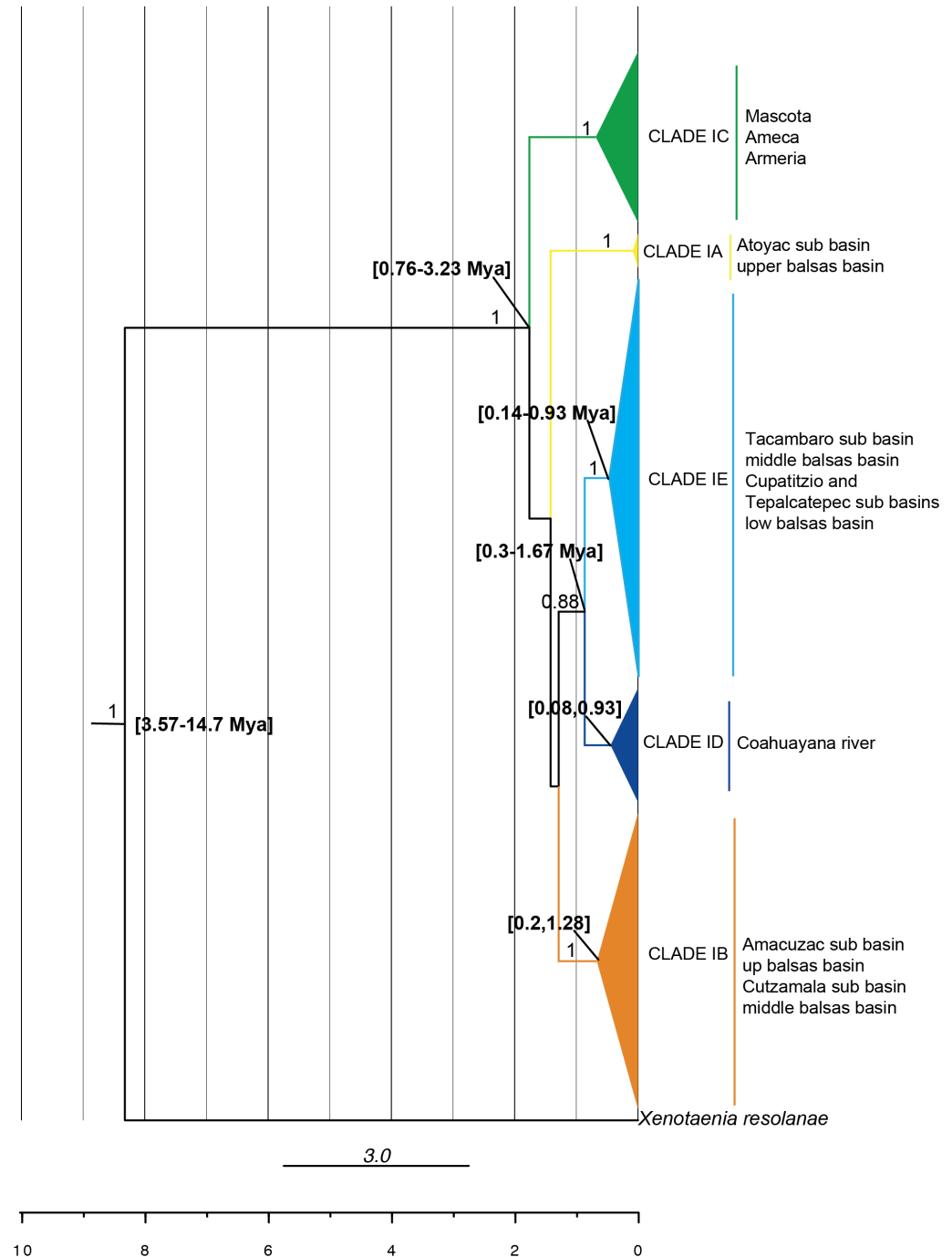


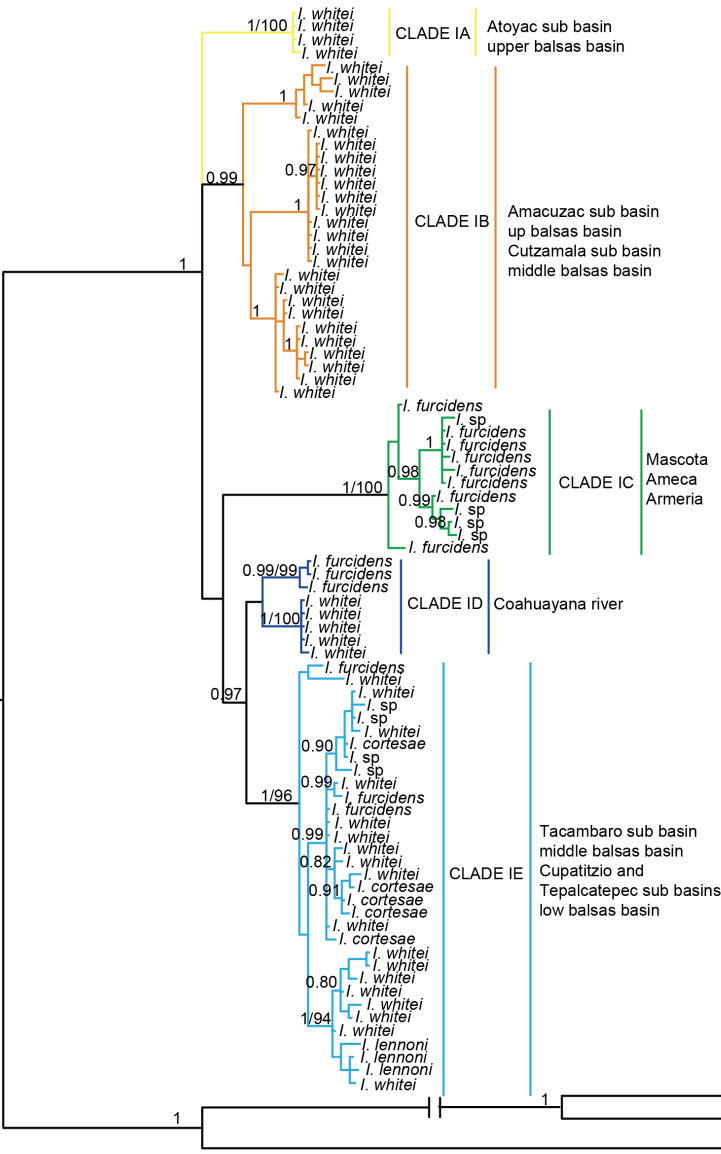
Divergence time between
Xenotaenia and *Ilyodon*
Miocene epoch

Divergence time between
Populations of Ameca and
Armeria river and the rest of
population

Between Pliocene and Pleistocene
epoch (1.99 Mya)

while the isolation of the
populations of the Coahuayana
river diverged of the populations
of Balsas basin in Pleistocene
epoch (0.98 Mya)





Cytb: 0.9-2.1%
 COI: 0.6-1.2%
 Between clades

| | CLADO IA | CLADO IB | CLADO IC | CLADO ID | CLADO IE |
|-----------------|------------|-------------|------------|-------------|-------------|
| <i>Cytb/COI</i> | | | | | |
| CLADO IA | 0.00/0.001 | 0.009 | 0.008 | 0.010 | 0.011 |
| CLADO IB | 0.016 | 0.005/0.005 | 0.009 | 0.012 | 0.012 |
| CLADO IC | 0.021 | 0.019 | 0.005/0.00 | 0.006 | 0.007 |
| CLADO ID | 0.014 | 0.015 | 0.019 | 0.003/0.003 | 0.006 |
| CLADO IE | 0.015 | 0.015 | 0.017 | 0.009 | 0.004/0.001 |

0.02 subs/site

Analyses of molecular variance with two different grouping (populations of each hydrological basin and populations according with recovered clades) for *cytb* and *COI*.

| Groups | df | Sum of squares | Variance components | % Variation |
|---|----|----------------|---------------------|-------------|
| <i>Cytb</i> (grouping with hydrological basin) | | | | |
| Among groups | 4 | 6.98 | 0.03 Va | 7.11 |
| Among populations within groups | 20 | 19.15 | 0.20 Vb | 41.24 |
| Within populations | 70 | 17.93 | 0.25 vc | 51.65 |
| F_{ST} : 0.48 ($p < 0.001$); F_{CT} : 0.07 (p ns); F_{SC} : 0.44 ($p < 0.001$) | | | | |
| <i>Cytb</i> (grouping with recovered clades) | | | | |
| Among groups | 4 | 212.75 | 3.00 Va | 72.09 |
| Among populations within groups | 19 | 48.84 | 0.62 Vb | 15.04 |
| Within populations | 66 | 35.45 | 0.53 vc | 12.88 |
| F_{ST} : 0.87 ($p < 0.001$); F_{CT} : 0.72 ($p < 0.001$); F_{SC} : 0.53 ($p < 0.001$) | | | | |
| <i>COI</i> (grouping with hydrological basin) | | | | |
| Among groups | 3 | 9.85 | 0.15 Va | 29.95 |
| Among populations within groups | 21 | 18.70 | 0.22 Vb | 41.85 |
| Within populations | 64 | 9.63 | 0.15 vc | 28.20 |
| F_{ST} : 0.71 ($p < 0.001$); F_{CT} : 0.29 ($p < 0.001$); F_{SC} : 0.59 ($p < 0.001$) | | | | |
| <i>COI</i> (grouping with recovered clades) | | | | |
| Among groups | 3 | 63.21 | 1.63 Va | 85.31 |
| Among populations within groups | 16 | 7.00 | 0.08 Vb | 4.52 |
| Within populations | 44 | 8.56 | 0.19 vc | 10.17 |
| F_{ST} : 0.89 ($p < 0.001$); F_{CT} : 0.85 ($p < 0.001$); F_{SC} : 0.30 ($p < 0.001$) | | | | |

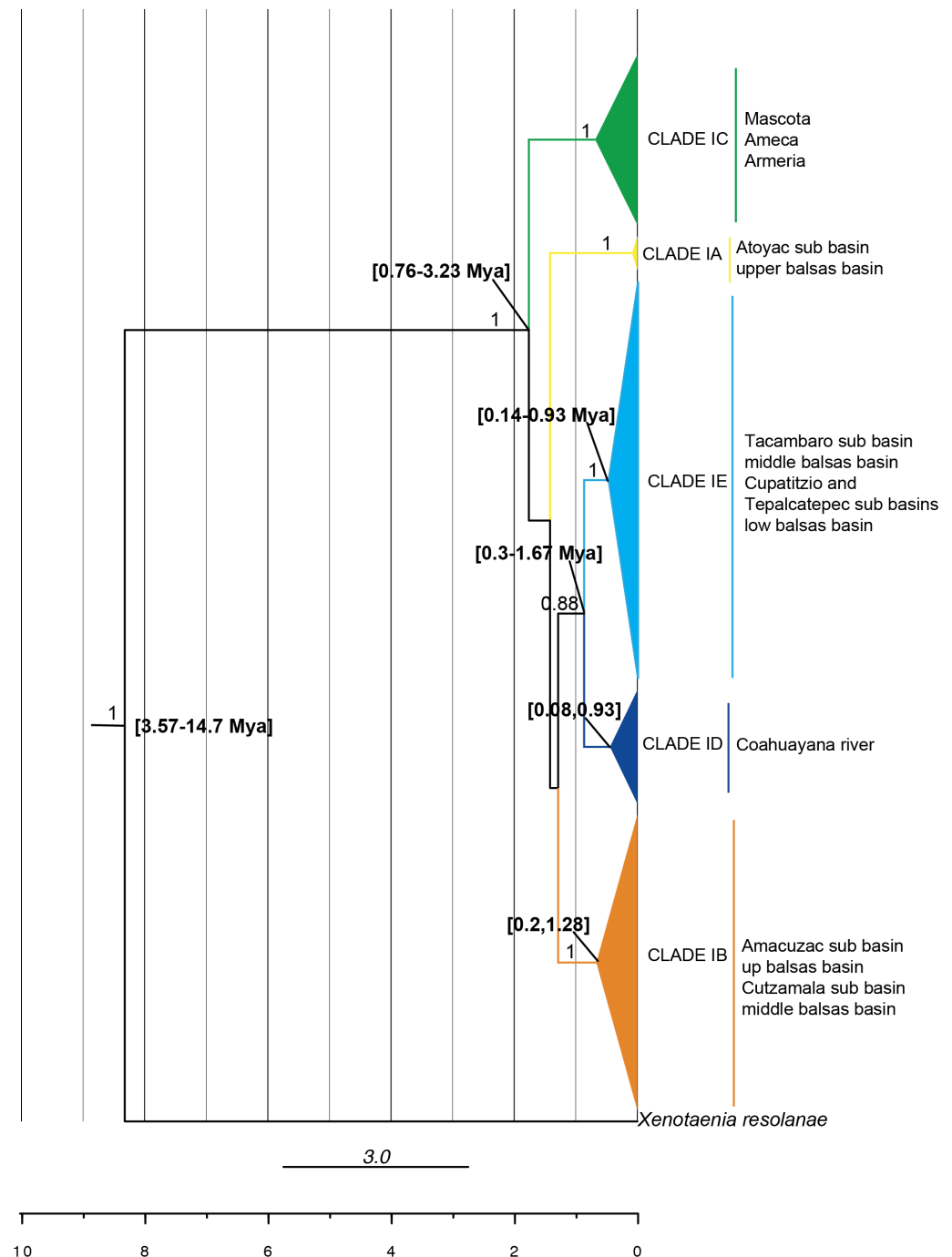
Intraspecific genetic distance
(0.4% Cytb, 0.1% COI)

I. xantusi, *I. lennoni* and *I. cortesae* are not valid species in this work, are only different trophic morphotypes:

- Mixed in topologies with *I. whitei* and *I. furcidens*
- Small genetic distances
- Shared haplotypes
- Same geographic distribution of *I. furcidens* and *I. whitei*

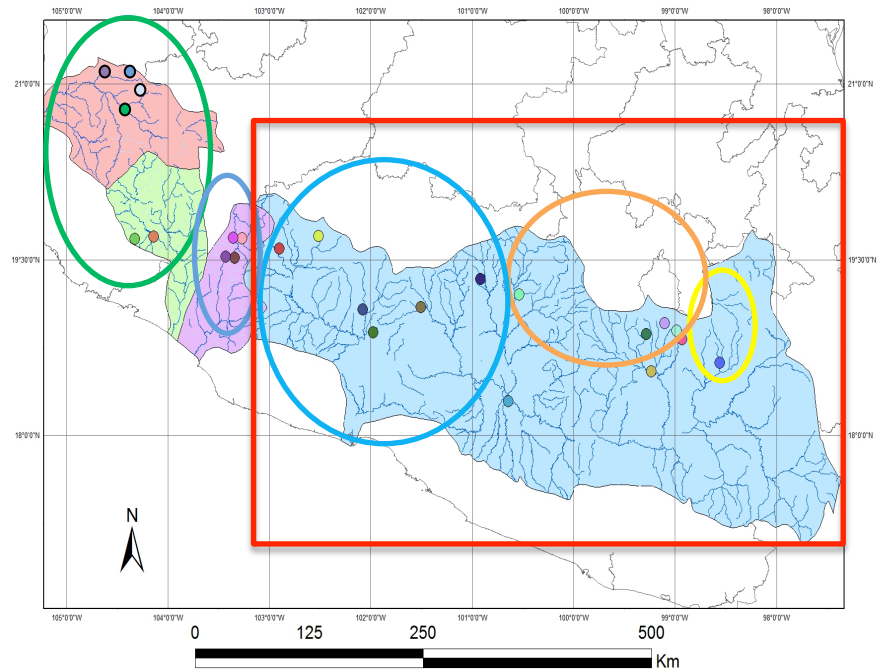
Recent diversification

- Pliocene and Pleistocene
- Clade IC with the rest coincide with the emergence of the Trans-Mexican-Volcanic Belt



Balsas basin

- High genetic structure
- High, middle and low Balsas basin
- Don't share haplotypes between them
- Independent clades
- Genetic distances (1.5-1.6% Cytb; 0.9-1.2% COI)
- Between Ameca, Armeria and Mascota (0.2-0.7% Cytb; 0% for COI).
- Other species as *Notropis*



Conservation

- Five clades with geographic correspondence
 - Five genetically different groups
 - Different units for conservation
 - *I. whitei* (IUCN, 2015)
 - *I. furcidents* (NOM-059)



Conclusions

- Five phylogenetic groups have geographic correspondence
- Diversification of genus *Ilyodon* in Pliocene and Pleistocene epoch
- *I. lennoni* and *I. cortesae* should be recognized as a synonym of *I. whitei*
- *I. amecae*, *I. furcidens* and *I. whitei*: Incomplete Lineages Sorting (ILS) or incipient species
- We recommended an extended work especially for populations of Balsas basin.

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¡ Thank you very much for your
attention !