Santiago Montero-Mendieta

Qualifications: Biologist
Specializations: Genomics, systematics, …
Current position: PhD student

ABOUT ME

2009-2013: Degree in Biology (University of Girona, Spain)

2013-2014: MSc in Biodiversity, focusing on Evolutionary Biology (University of Barcelona, Spain)

2015-Present: PhD Student, Estación Biológica de Doñana (CSIC) Seville, Spain (1 year and a half)

GOBIERNO DE ESPAÑA
MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD
CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

Estación Biológica Doñana CSIC

A GENOMIC VIEW ON THE DIVERSIFICATION OF NEOTROPICAL FROGS

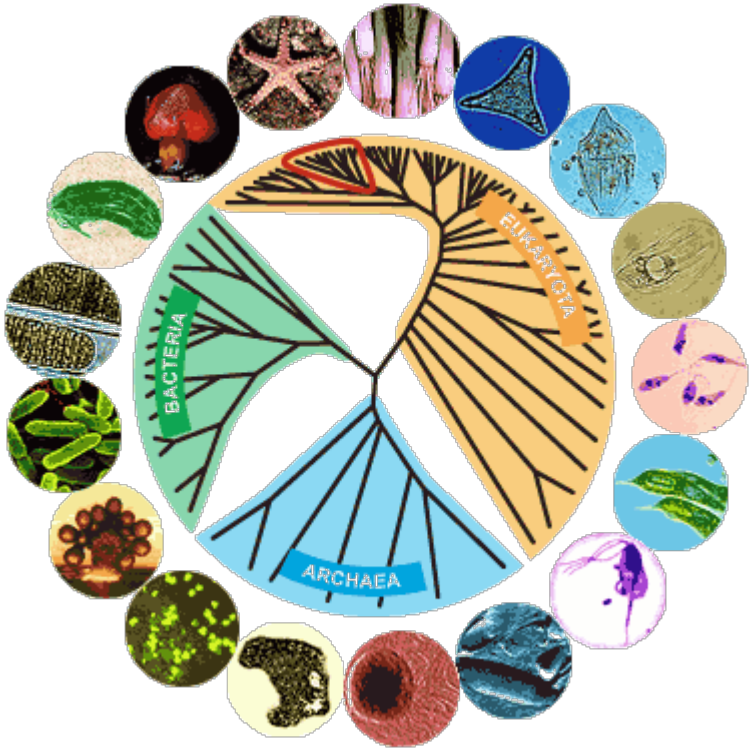
(provisional title)

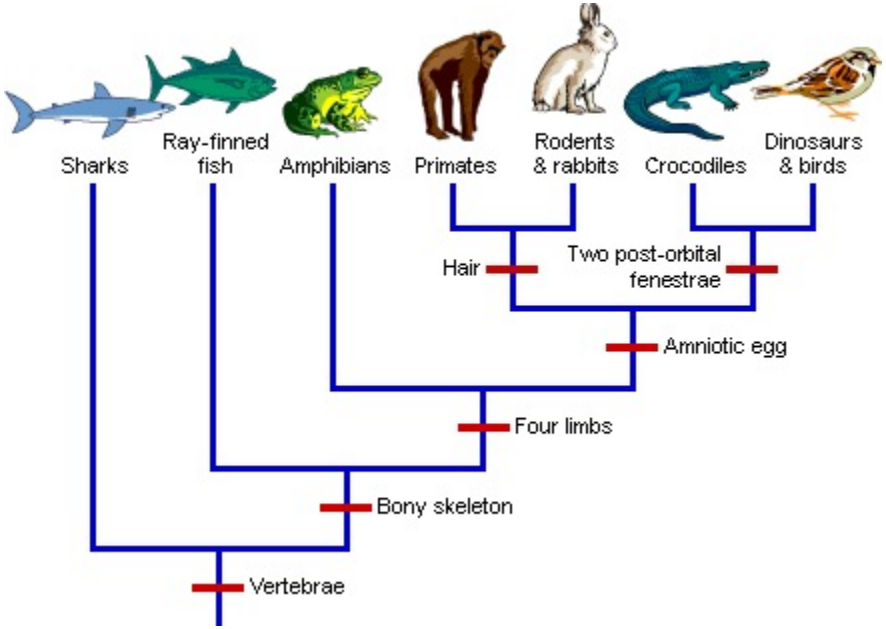
Main advisor: Carles Vilà

Collaborators: Jennifer Leonard, Matthew Webster, José Manuel Padial & Ignacio De la Riva

The history of life

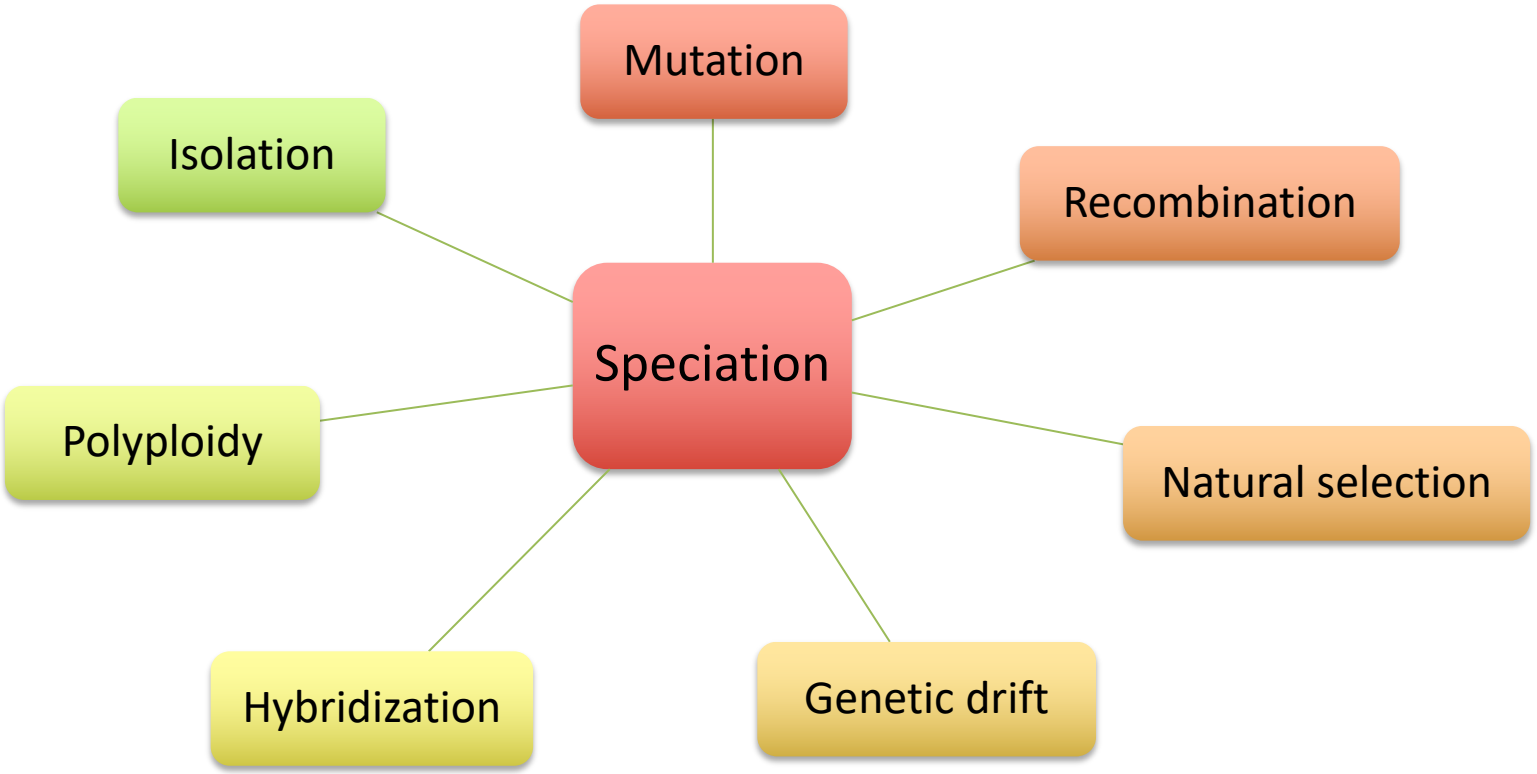
The theory of evolution is based on the idea that all species are related and gradually change over time.





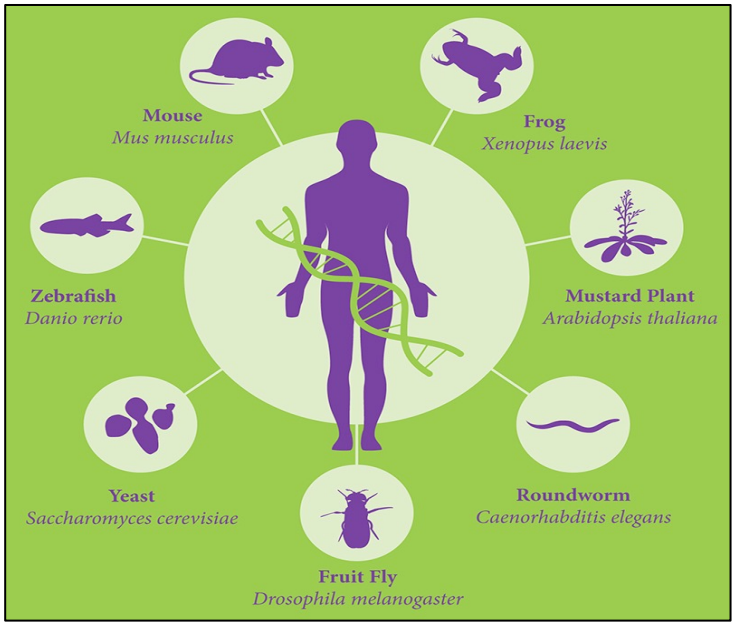
What is speciation?

The formation of new and distinct species in the course of evolution



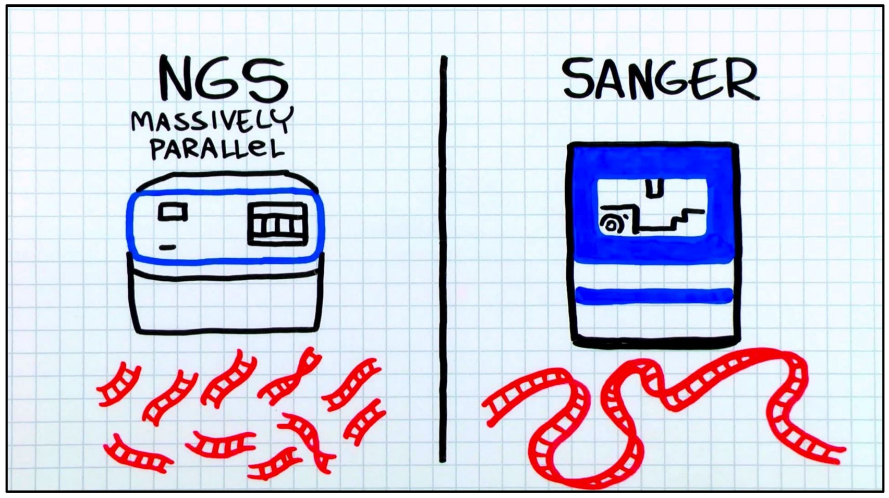
Model & non-model organisms

High-throughput sequencing (e.g. Illumina) makes non-model organisms increasingly accessible for speciation studies, mainly through proteomics



Speciation genomics

Large amounts of orthologous loci can be obtained, allowing the use of less individuals



LIMITED POWER

Pre-selected markers are used

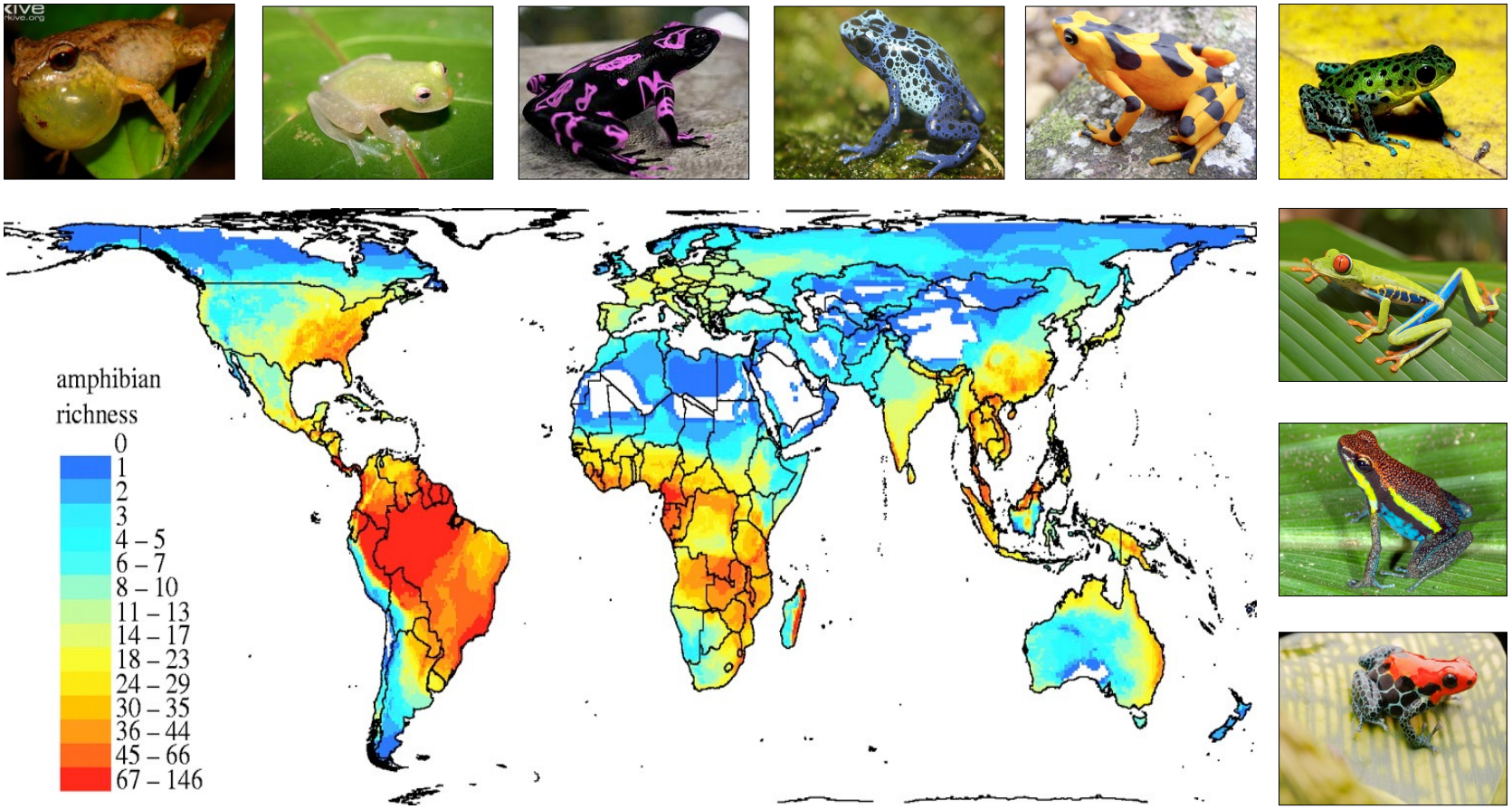
Need of sampling multiple individuals

1. It allows finding genes involved in speciation
2. It allows finding genes homogenized by gene-flow (or those that resist introgression)
3. It allows finding genes related to adaptation

Humans & Neanderthals mated in the past

Green et al. (2010)

Neotropical amphibians

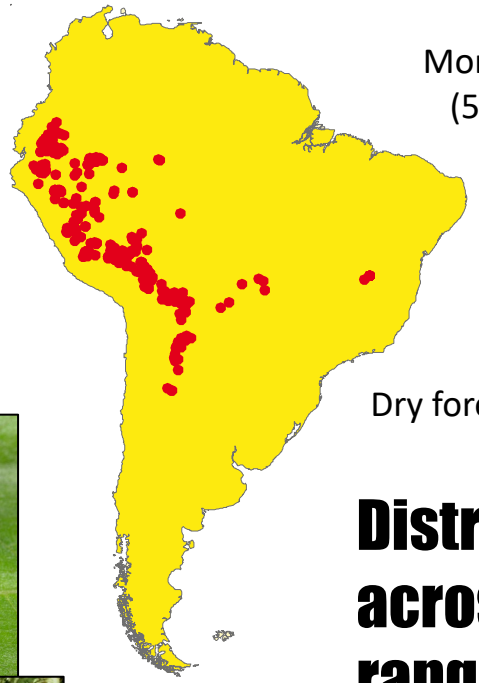


~ 50% of world's amphibians

Our study model

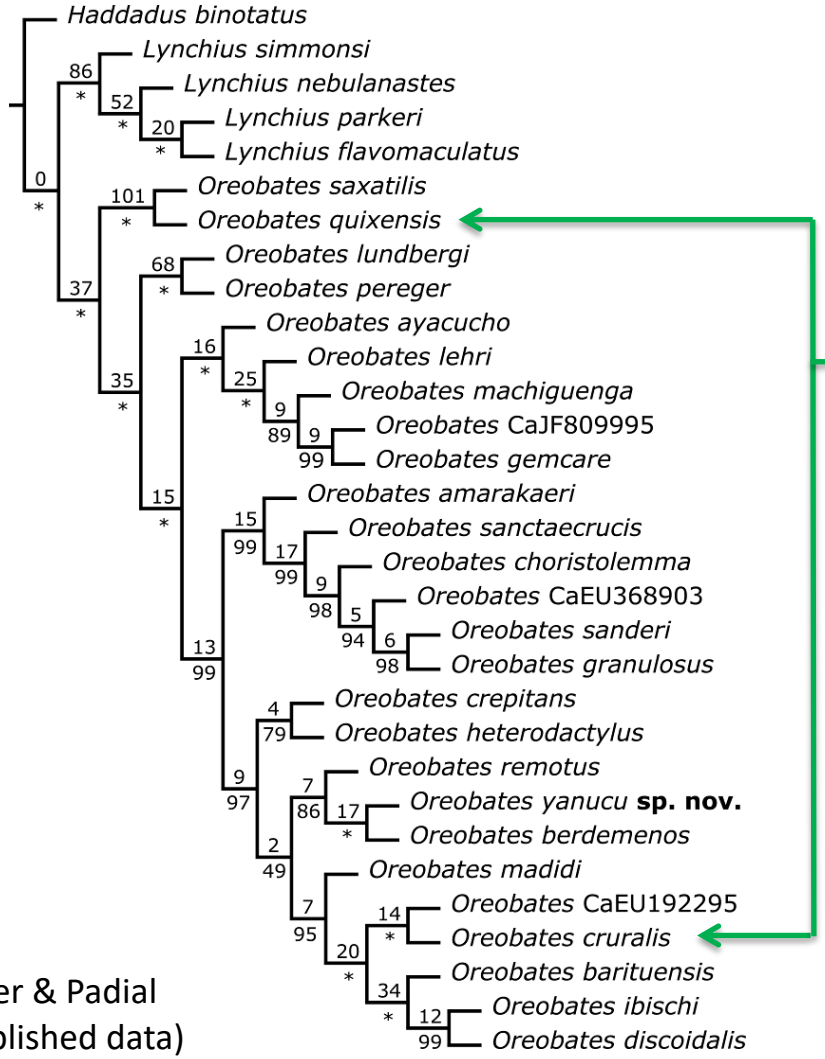
- Frogs of the genus **Oreobates**

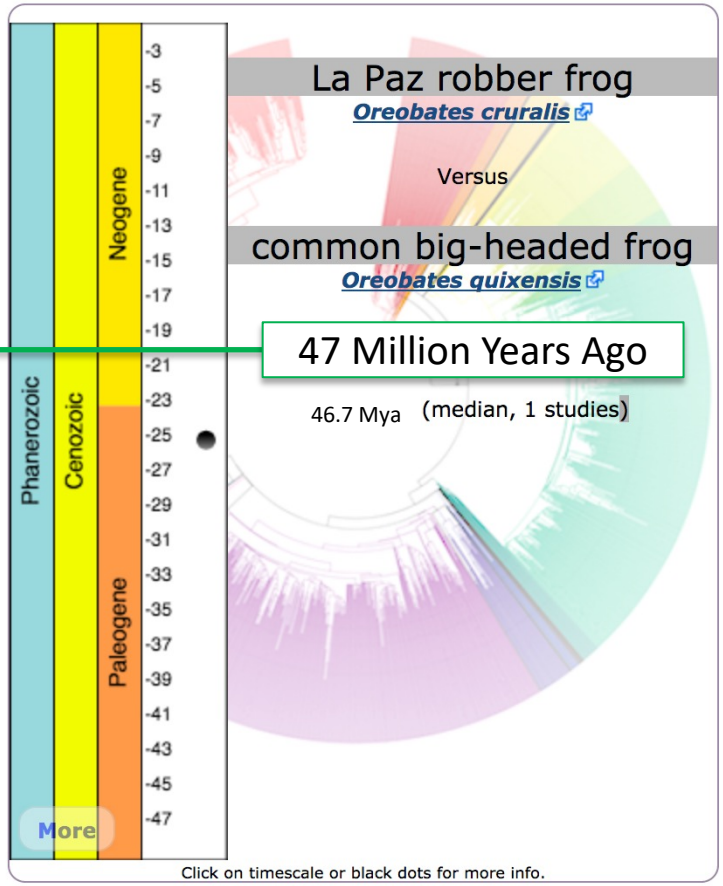
24 species up to date



Distributed across a wide range of habitats and altitudes

Still little is known





?

Kohler & Padial
(unpublished data)

The best non-model

>>> Challenge effect <<<

Extremely difficult to sample

- Difficult to find
- Few museums have specimens
- Logistic problems (permits)

Few genomic data available in frogs

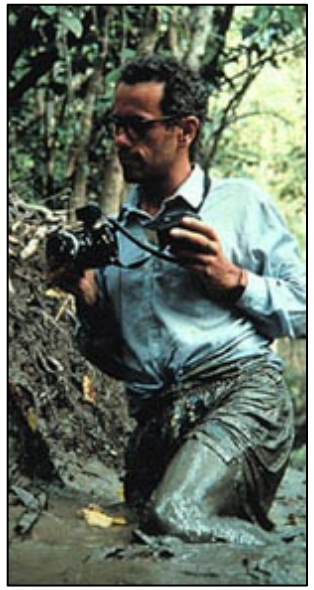
- *Xenopus tropicalis* (206.6 Mya)
- *Nanorana parkeri* (156.0 Mya)

Some species have been only found once (by our collaborators)

Why did we chose Oreobates?

- *Oreobates amarakaeri* (Padial et al., 2012)
- *Oreobates ayacucho* (Lehr, 2007)
- *Oreobates barituensis* (Vaira & Ferrari, 2008)
- *Oreobates berdemenos* (Pereyra et al., 2014)
- *Oreobates choristolemma* (Harvey & Sheehy, 2005)
- *Oreobates crepitans* (Bokermann, 1965)
- *Oreobates cruralis* (Boulenger, 1902)
- *Oreobates discoidalis* (Peracca, 1895)
- *Oreobates gemcare* (Padial et al., 2012)
- *Oreobates granulosus* (Boulenger, 1902)
- *Oreobates heterodactylus* (Miranda-Ribeiro, 1937)
- *Oreobates ibischi* (Reichle, et al. 2001)
- *Oreobates lehri* (Padial et al., 2007)
- *Oreobates lundbergi* (Lehr, 2005)
- *Oreobates machiguenga* (Padial et al., 2012)
- *Oreobates madidi* (Padial et al., 2005)
- *Oreobates pereger* (Lynch, 1975)
- *Oreobates quixensis* (Jiménez de la Espada, 1872)
- *Oreobates remotus* (Teixeira et al., 2012)
- *Oreobates sanctaecrucis* (Harvey & Keck, 1995)
- *Oreobates sanderi* (Padial, et al., 2005)
- *Oreobates saxatilis* (Duellman, 1990)
- *Oreobates yanucu* (Kohler & Padial 2016)
- *Oreobates zongoensis* (Reichle & Köhler, 1997)

José Manuel Padial



Ignacio De la Riva

Our collaborators are experts on these frogs

We have access to (almost) all the Oreobates species

Research goals

To study evolution rates, demographic history and adaptation patterns on the frogs of the genus Oreobates

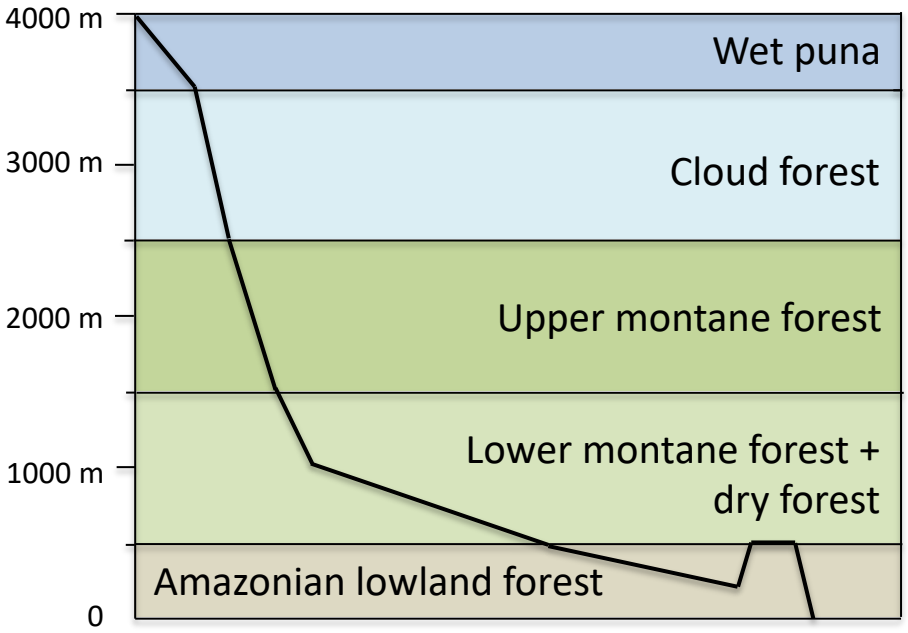




1. **Phylogenomics**: genetic relationship among Oreobates
2. **Evolutionary history**: study variation in evolution rates
3. **Demographic history**: track demographic changes through time and correspondence with habitat changes
4. **Adaptation**: identify genes that have been differentiated between populations (adaptation)

1st stage : phylogenomics

Andes mountain diagram



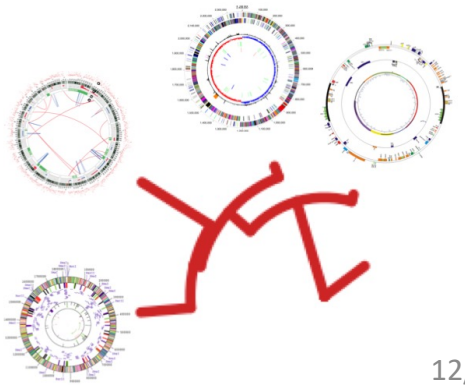
Goal: to build a highly supported tree for downstream analysis

When the genus Oreobates was originated? How many Oreobates species?

How many colonization events to lowland rainforest have occurred?

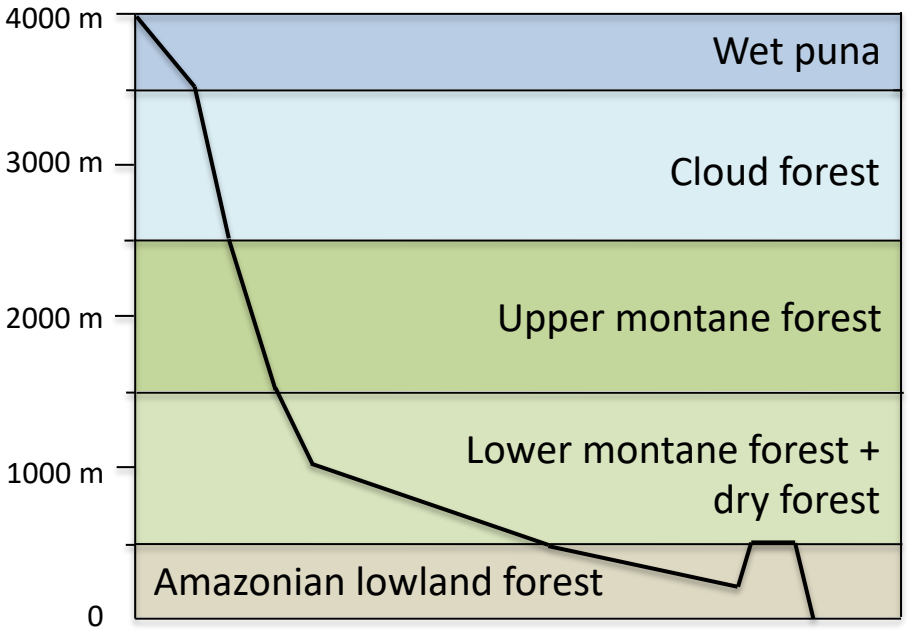
H-1: Oreobates emerged on the Andes highlands

Fact: Now there are species living on both highlands and lowlands



2nd stage: evolutionary history

Andes mountain diagram



Goal: study the variation in the evolution rate of Oreobates

Is the evolution rate lower in the highland species?

Global Ecology and Biogeography, (Global Ecol. Biogeogr.) (2015) **24**, 804–813

RESEARCH PAPER

A test of the integrated evolutionary speed hypothesis in a Neotropical amphibian radiation

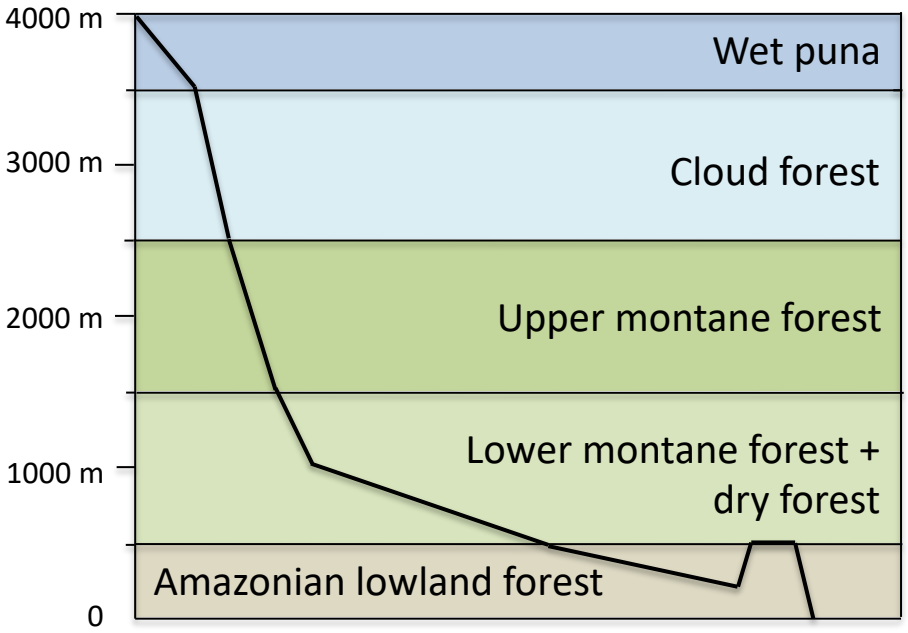
Álvaro Dugo-Cota[1*], Santiago Castroviejo-Fisher[2,3], Carles Vilà[1] and Alejandro Gonzalez-Voyer[1,4,5]

H-2: Ectotherm metabolism slows down at low temperatures

Fact: Previous studies in glass-frogs proved a reduction in the rate of evolution in highland environments

3rd stage: demographic history

Andes mountain diagram



Goal: study the effect of the past environmental conditions on the Oreobates demography

Do highland species show different demographic trends compared to lowland?

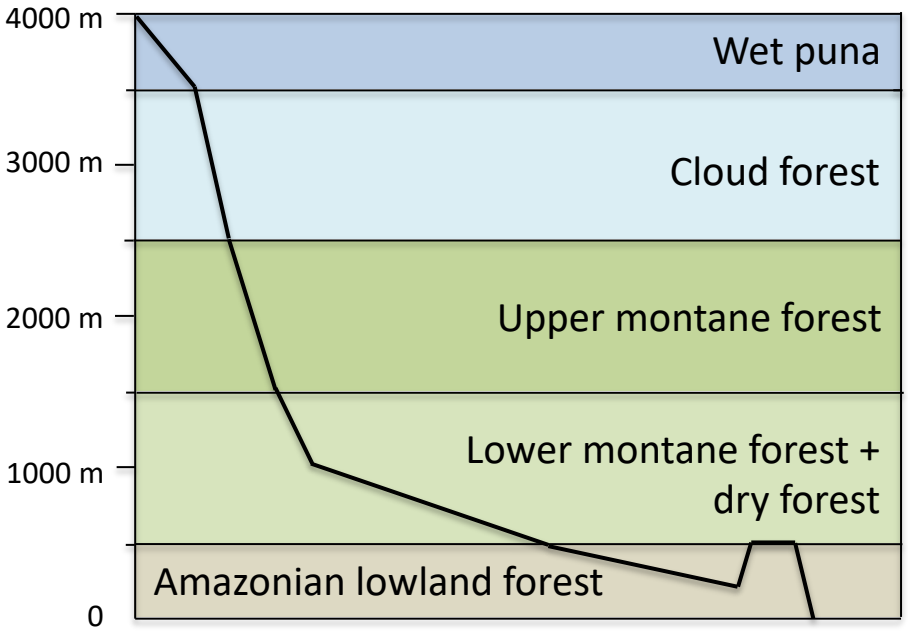
Is there any hybridization between diverging linages living on the lowlands?

H-3: Species with similar habitat requirements will show parallel demographic changes during Pleistocene climate changes

O. quixensis *O. saxatilis*

4th stage: study of adaptation

Andes mountain diagram



Goal: study the genomic signatures of speciation

Are there any genes related to adaptation to highlands?

Are there any genes related to adaptation to dry forest?

H-4: Genomic regions associated with adaptation to environment should show larger genetic divergences

Fact: Some Oreobates inhabit a wide diversity of habitats

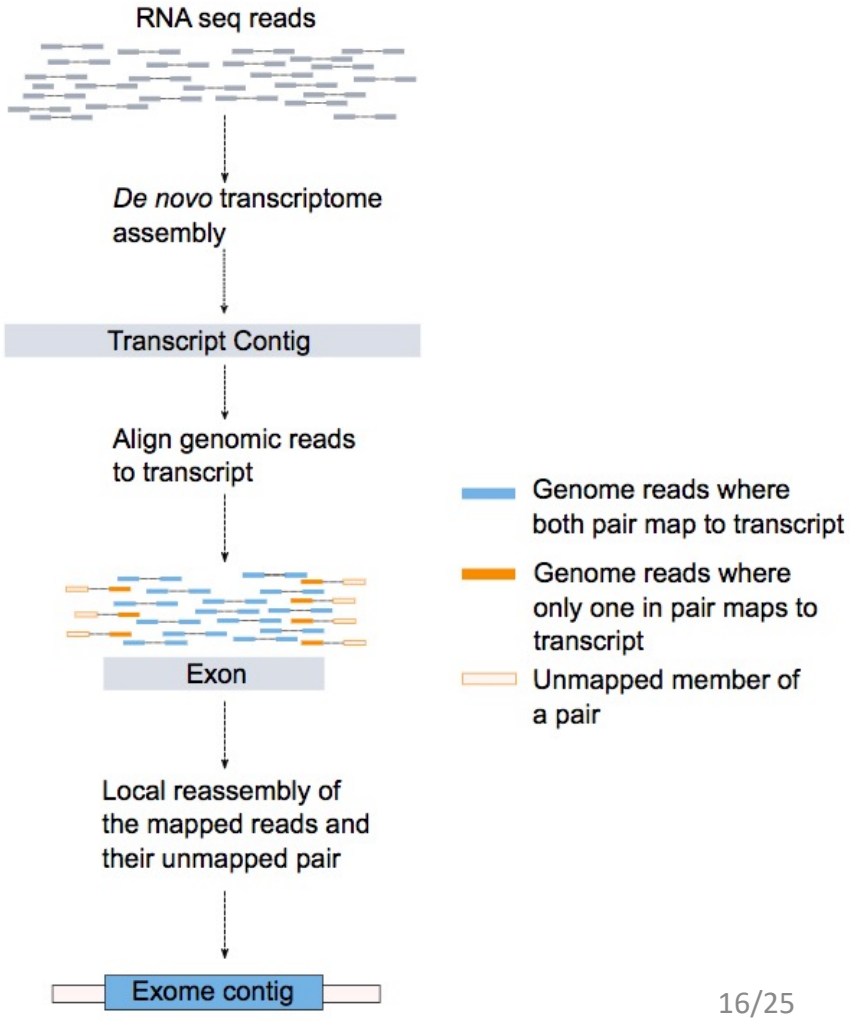
O. cruralis

Our initial idea

- 1st : Transcriptome sequencing (as a reference)
- 2nd : Whole genome sequencing (for the others)
- 3rd : Exome assembly
- 4th : SNP detection and analyzing data

DRAWBACK: big waste

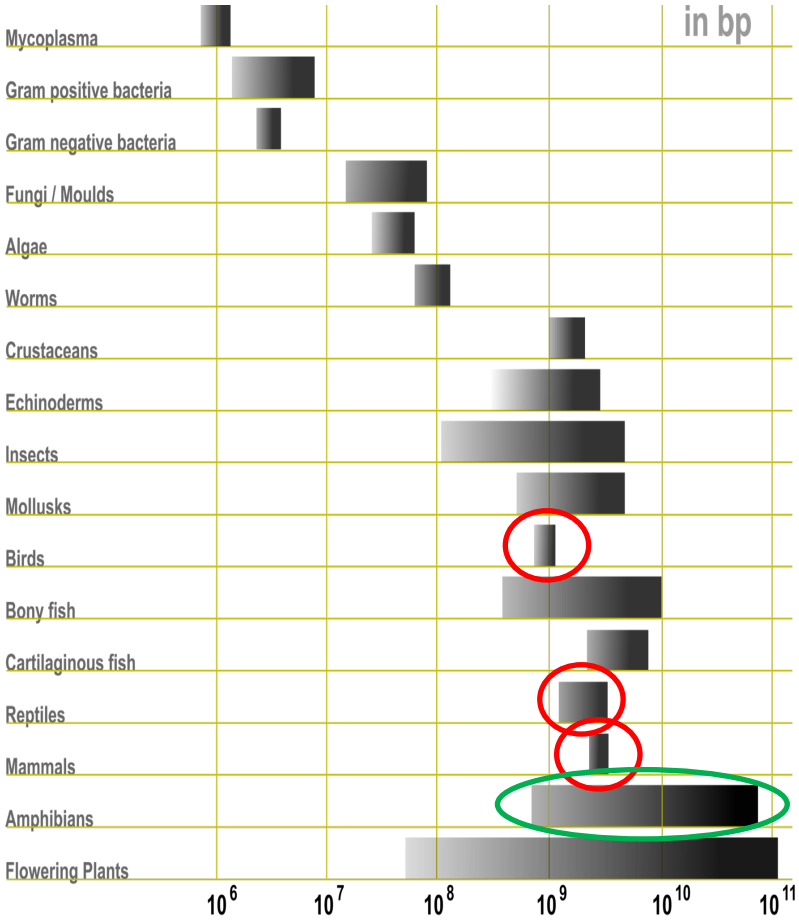
NEED OF: genome size



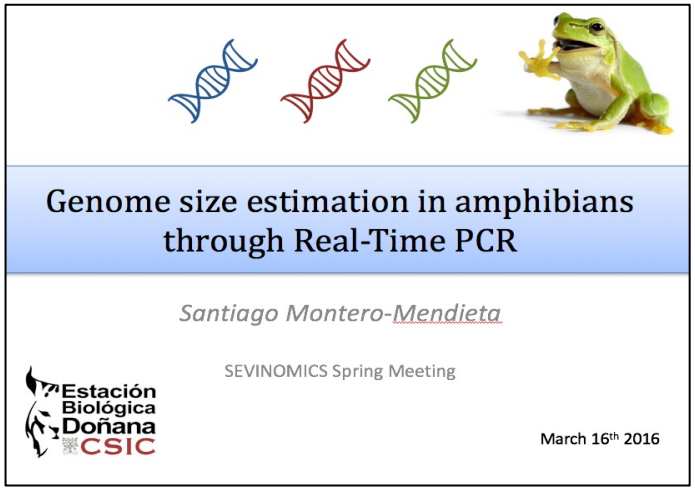
Adapted from Lamichhaney et al. (2012)

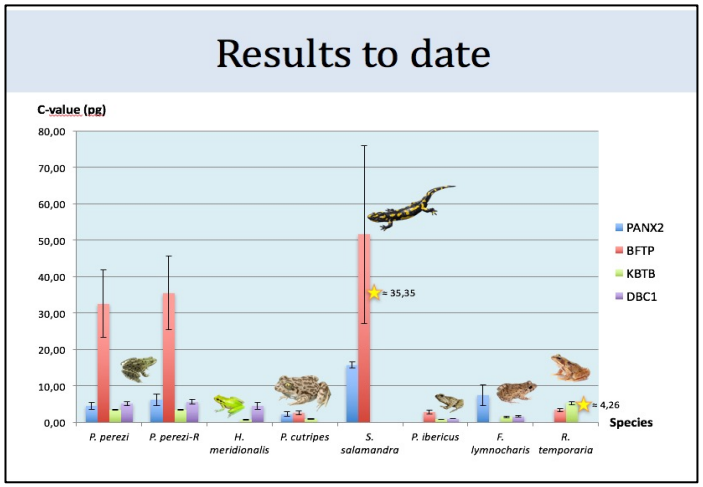
Amphibians have big genomes

The C-value Enigma



Previous work

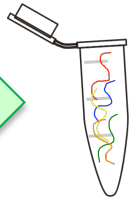




Reduced representation of the genome: *transcriptome*

How to do it?

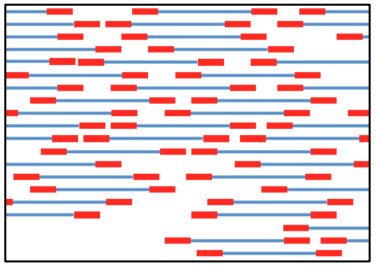
Oreobates cruralis



Total RNA

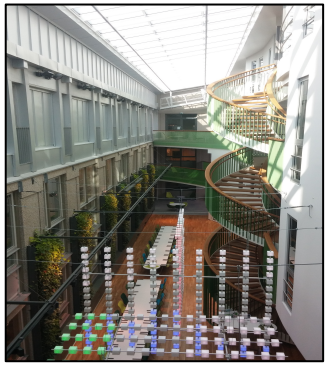
- ✓ Liver
- ✓ Intestine
- ✓ Spleen

Transcriptome sequencing



Bioinformatics analysis

De-novo transcriptome assembly

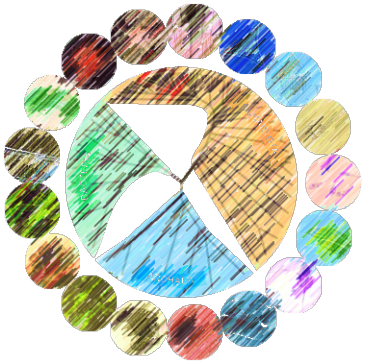


Uppsala University

NOW

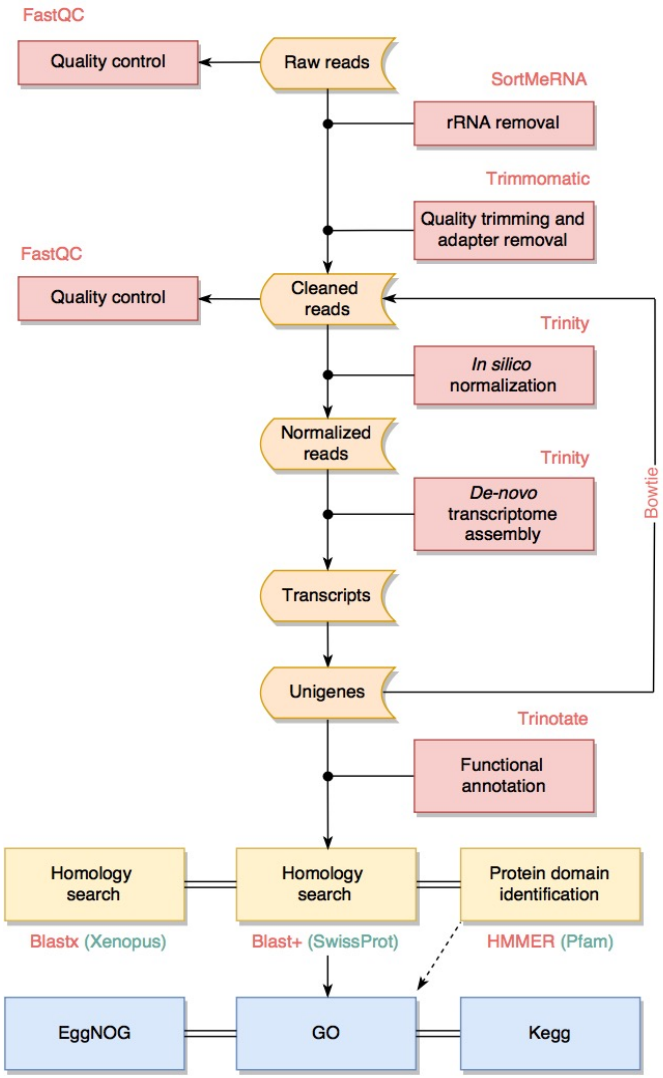
Transcriptome-based exon capture

(on-going)



Phylogenomics & more

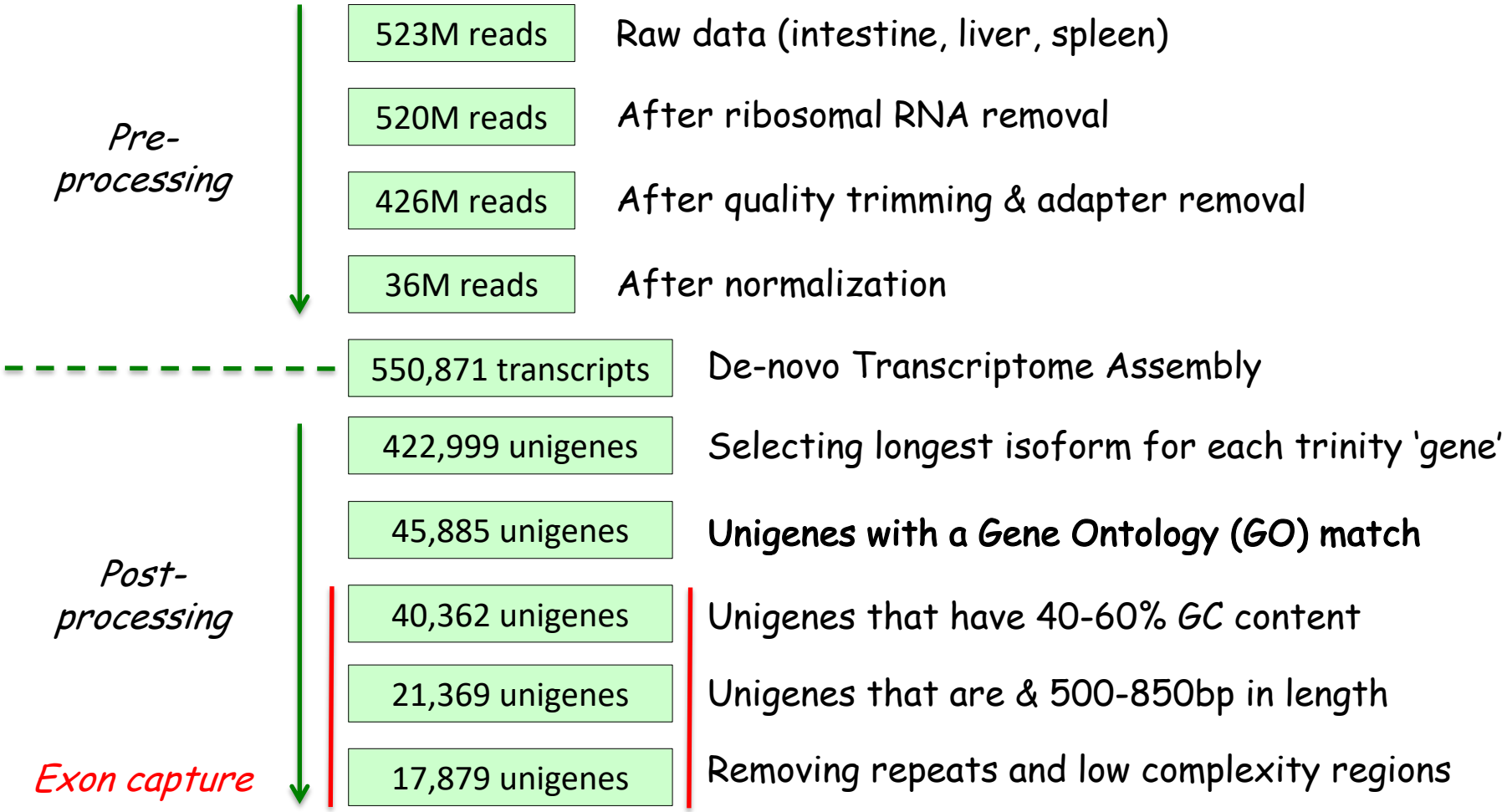
Transcriptome workflow



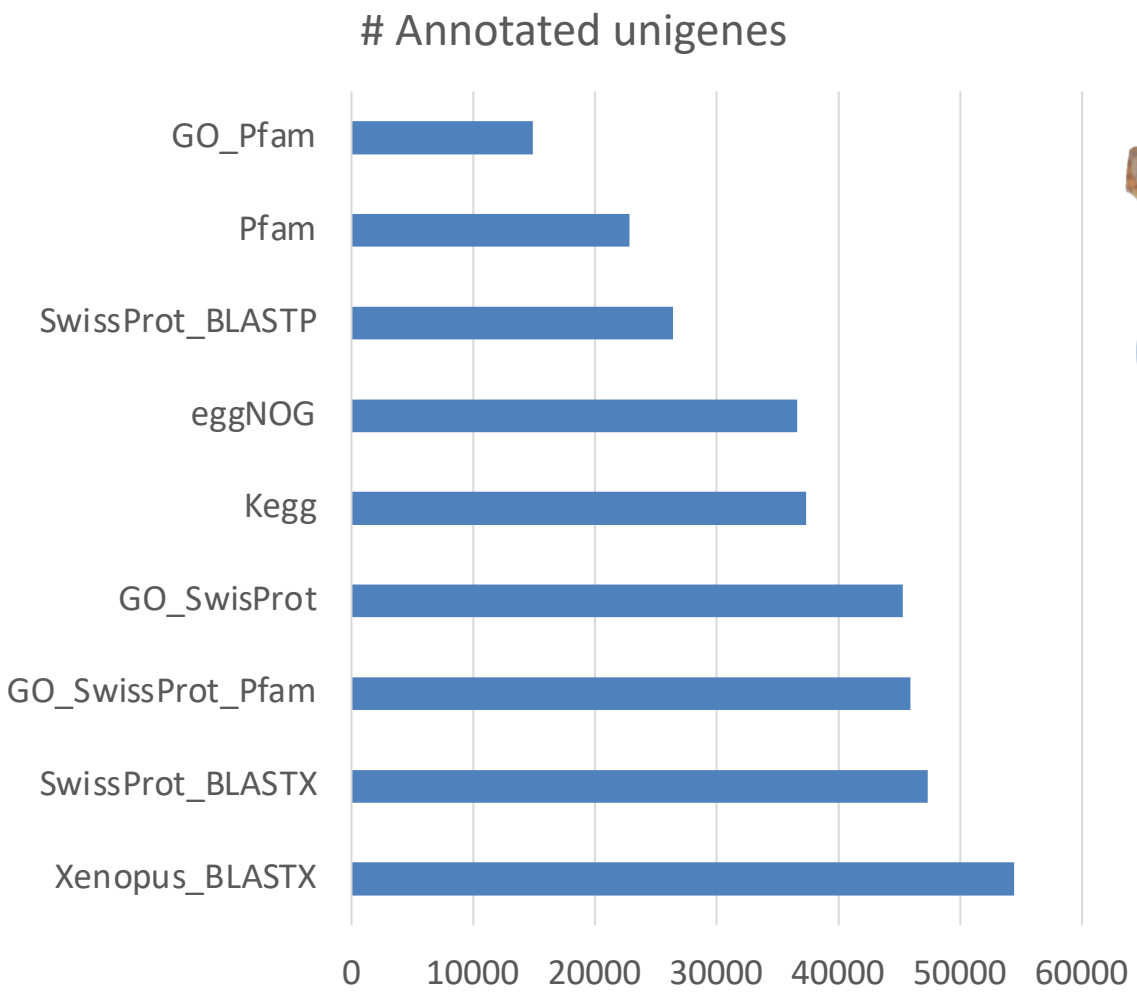
Quick guide to build de-novo assemblies

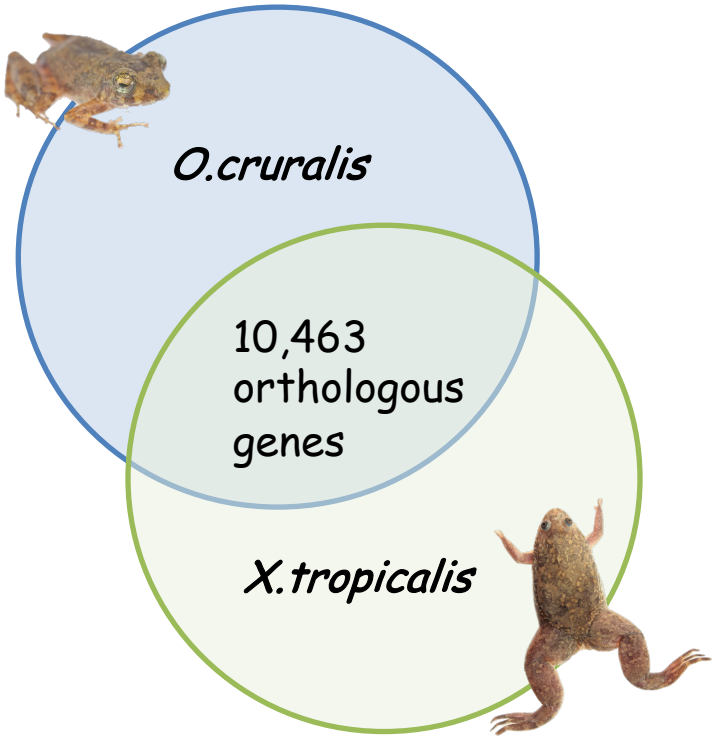
1. Get raw reads (RNAseq data)
2. Quality control [FastQC]
3. Ribosomal RNA removal [SortMeRNA]
4. Quality trimming & adapter removal [Trimmomatic]
5. Quality control (again) [FastQC]
6. In silico normalization [Trinity]
7. Merge data (when multiple tissues per sample)
8. In silico normalization (again) [Trinity]
9. De-novo transcriptome assembly [Trinity]
    9.1. Assembly validation [Bowtie]
10. Functional annotation [Trinotate]

Transcriptome results (I)



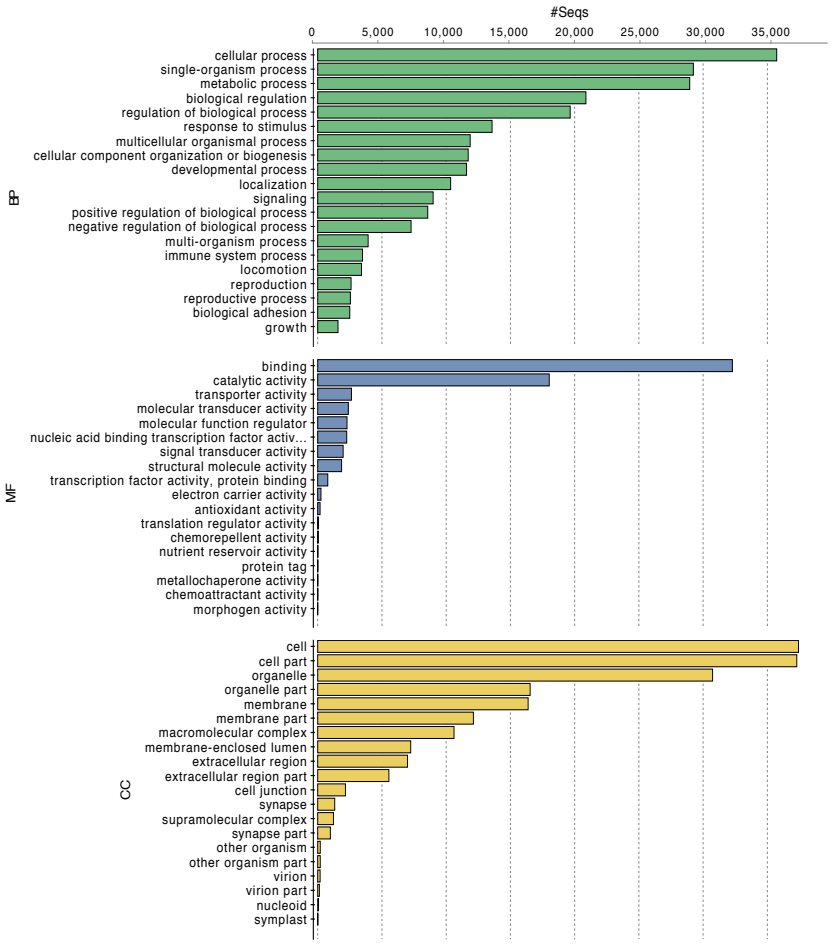
Transcriptome results (II)





Transcriptome results (III)

Gene Ontology (GO) categories



Percentage of unigenes

→ **Biological process**

Cellular process..... 78%
Single-organism process..... 64%
Metabolic process..... 63%

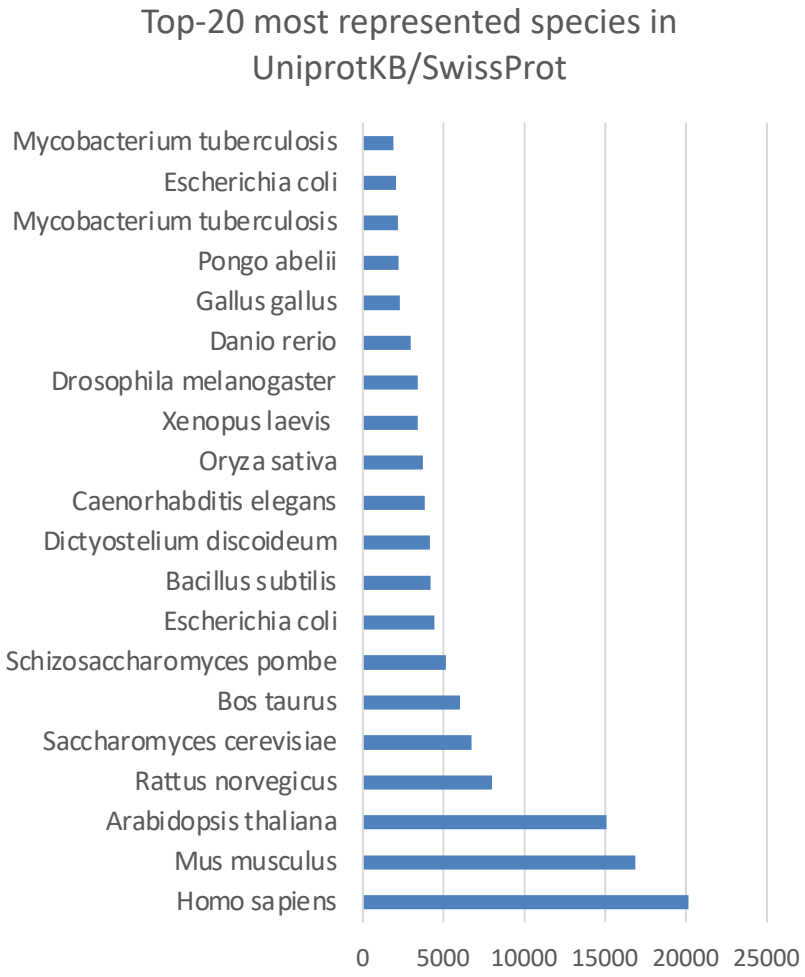
→ **Molecular function**

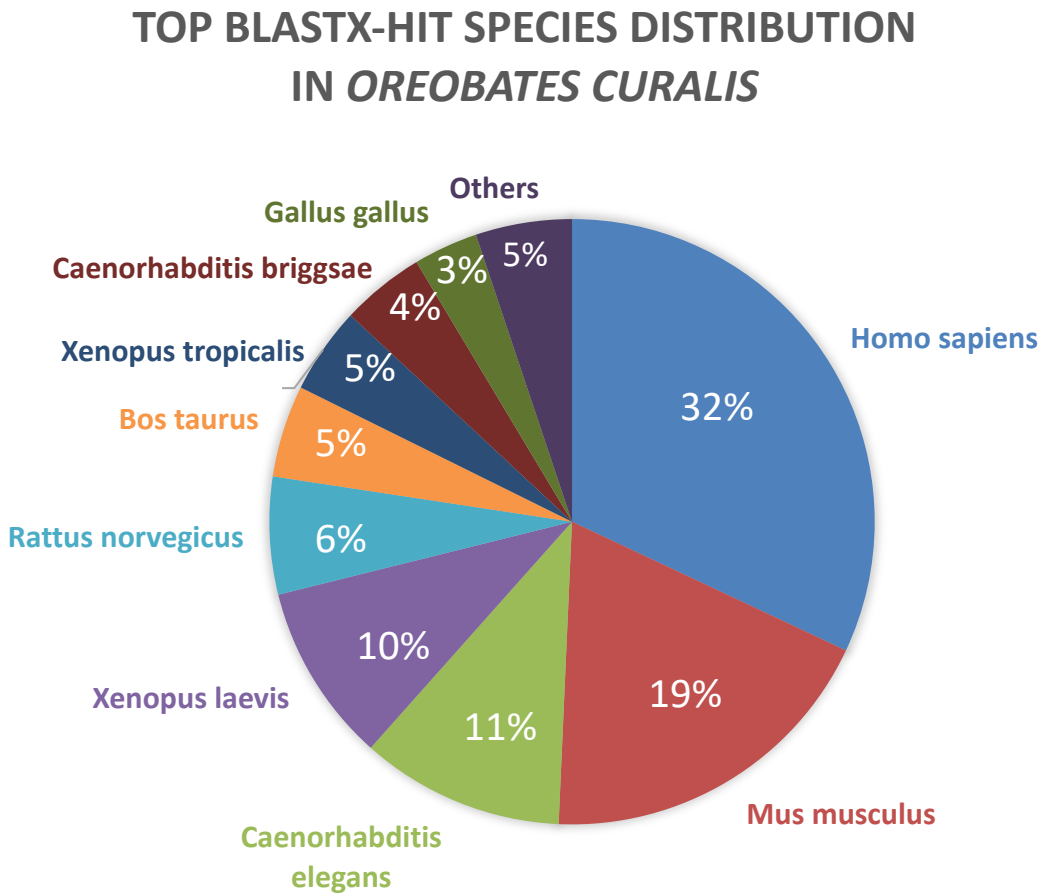
Binding..... 70%
Catalytic activity..... 39%

→ **Cellular component**

Cell..... 82%
Cell part..... 81%
Organelle..... 67%

Transcriptome results (IV)





So, what is next?

MOLECULAR ECOLOGY
RESOURCES
Molecular Ecology Resources (2016) 16, 1069–1083 doi: 10.1111/1755-0998.12541

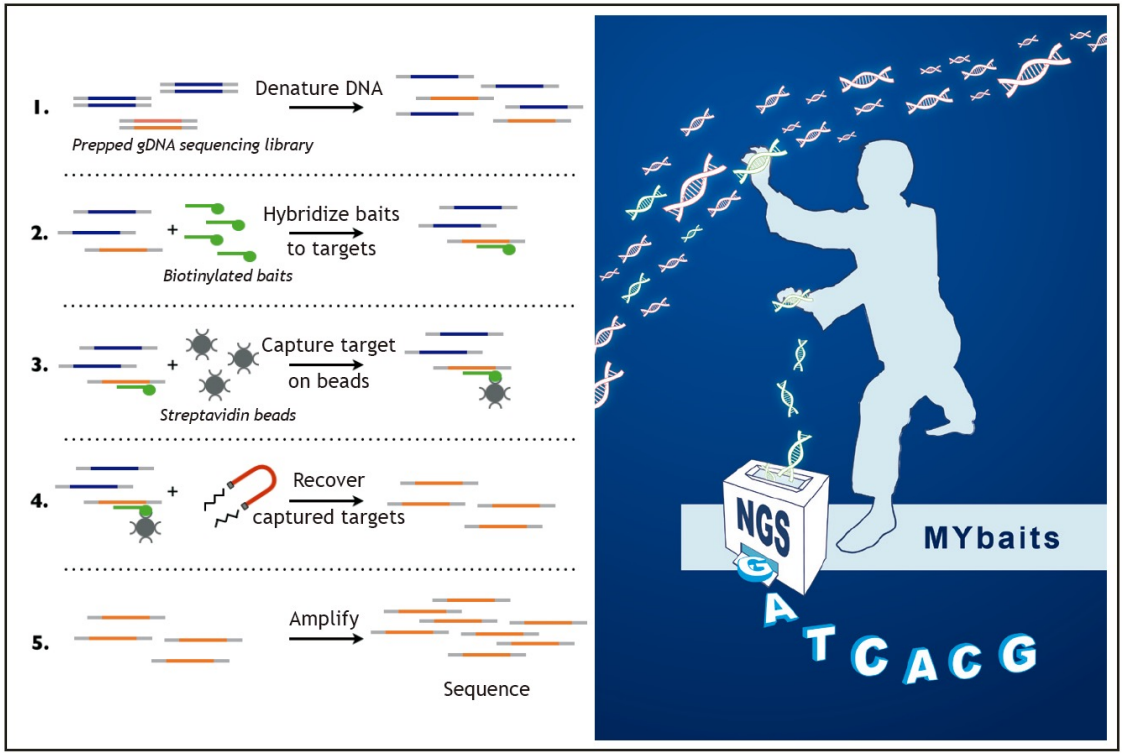
Transcriptome-based exon capture

SPECIAL ISSUE: SEQUENCE CAPTURE

An evaluation of transcriptome-based exon capture for frog phylogenomics across multiple scales of divergence (Class: Amphibia, Order: Anura)

We are using the 17,879 unigene sequences from *O. cruralis* to design capture probes for all other Oreobates species.

Orthologous genes will be identified and used to test initial hypothesis



" We gotta catch 'em all ! "

Thanks for your attention!

QUESTIONS?