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# Characterizing the genetic diversity of immune genes in a non-native population of American Bullfrogs in Humboldt County, California

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## Background

- American Bullfrogs (*Lithobates catesbeianus*) are native to the East Coast of the U.S.
- These frogs are highly invasive and have successfully colonized different habitats all over the world
- They have a number of negative effects on the ecosystem they colonize:
  - Introduce new diseases
  - Cause decline in native frog populations.
  - Outcompete native frog populations for prey items
- Previous studies have focused on the ecological impact of bullfrogs



Figure 1. An adult American bullfrog ([http://www.californiaherps.com/frogs/images/rcatesbeianadna\\_b707.jpg](http://www.californiaherps.com/frogs/images/rcatesbeianadna_b707.jpg))

## Study population and focal gene

- 16 Bullfrog metamorphs were collected from Mad River, CA
- Focal gene: Major Histocompatibility Complex (MHC) class II beta chain, exon 2
  - Highly variable gene involved in acquired immunity in jawed vertebrates
  - Gene encodes a transmembrane receptor (Fig. 2)

- Having different alleles can influence disease susceptibility to viral, bacterial, and fungal infections in frogs (e.g., Savage and Zamudio 2011, Barribeau et al. 2008, Teacher et al. 2009)

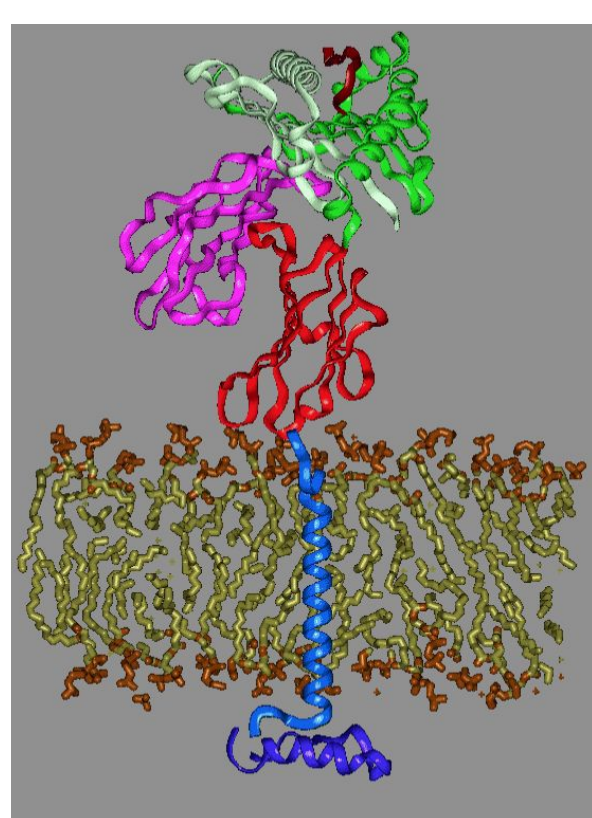
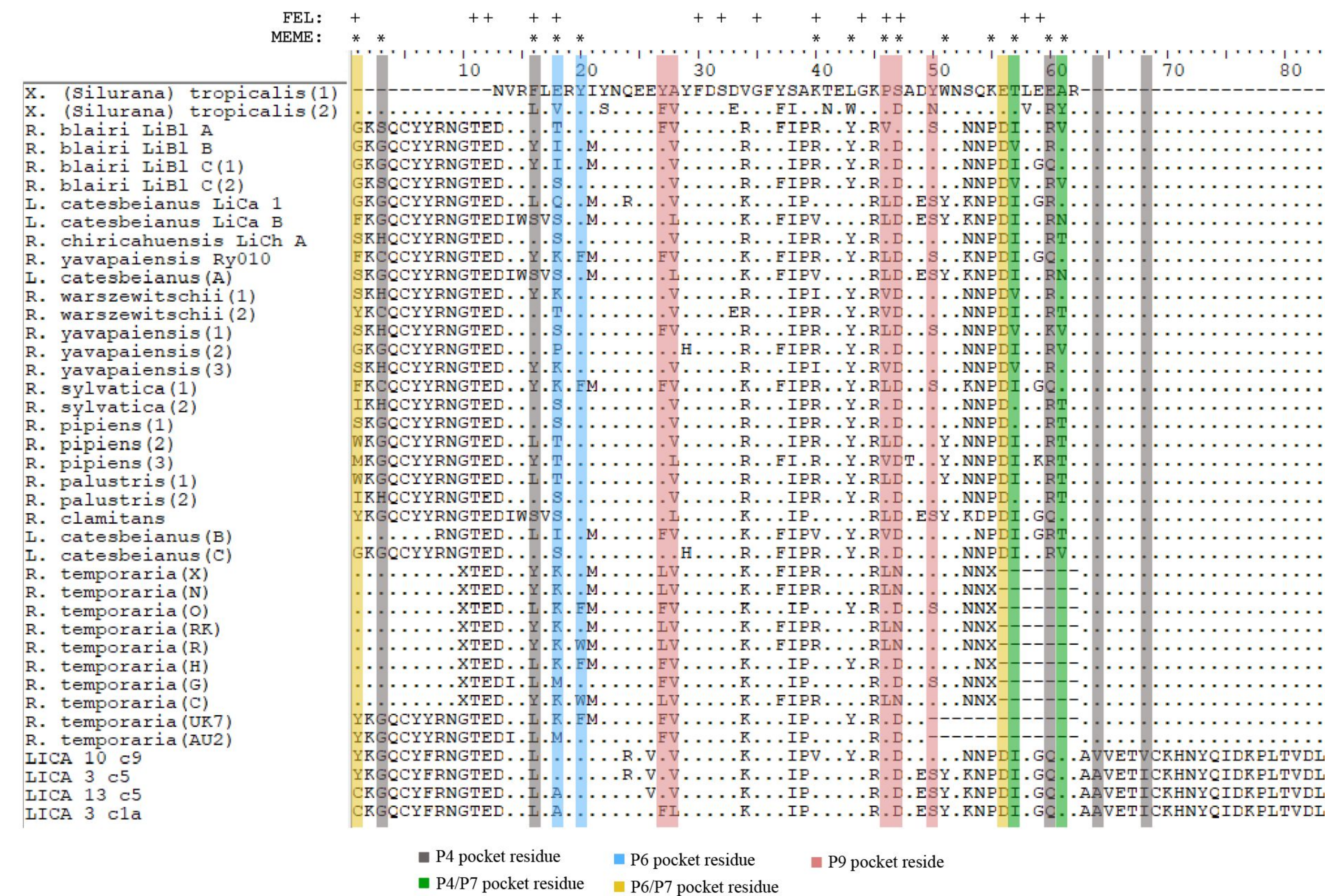


Figure 2. Schematic of an MHC protein embedded in a cell membrane (adapted from <http://www.cryst.bbk.ac.uk/ppsp97/assignments/projects/coadwell/MHCSTFU1.HTM>)

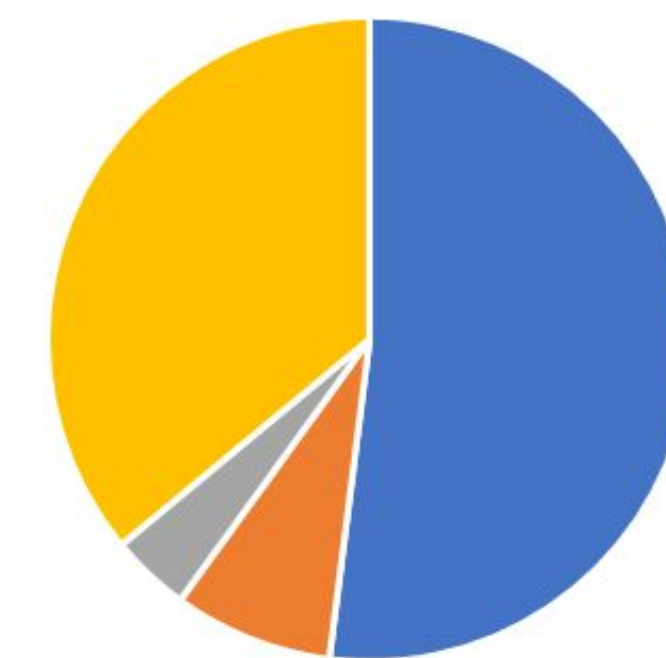
## Methods

- Tissue collection**
  - Whole frogs stored in ethanol and later liver tissue was dissected out
- Gene isolation**
  - DNA was isolated from tissues using a silica-column kit and MHC Class II B1 locus was amplified using PCR (Mulder et al. 2017)
  - PCR product was purified and cloned using standard T/A cloning vector system (Promega Corporation)
  - Eight bacterial colonies per individual were sequenced
- DNA sequence analysis**
  - Sequences were cleaned, edited, and aligned using MEGA and Snapgene viewer
  - A maximum likelihood tree using nucleotide data (with 100 bootstrap replicates) was built with MEGA
  - Allele frequency pie charts generated in Excel
- Selection on codons was estimated using MEME/FEL (datamonkey.org)**

## MHC class II B1 chain exhibits selection on its peptide-binding residues



## Allele frequencies are skewed in our focal population



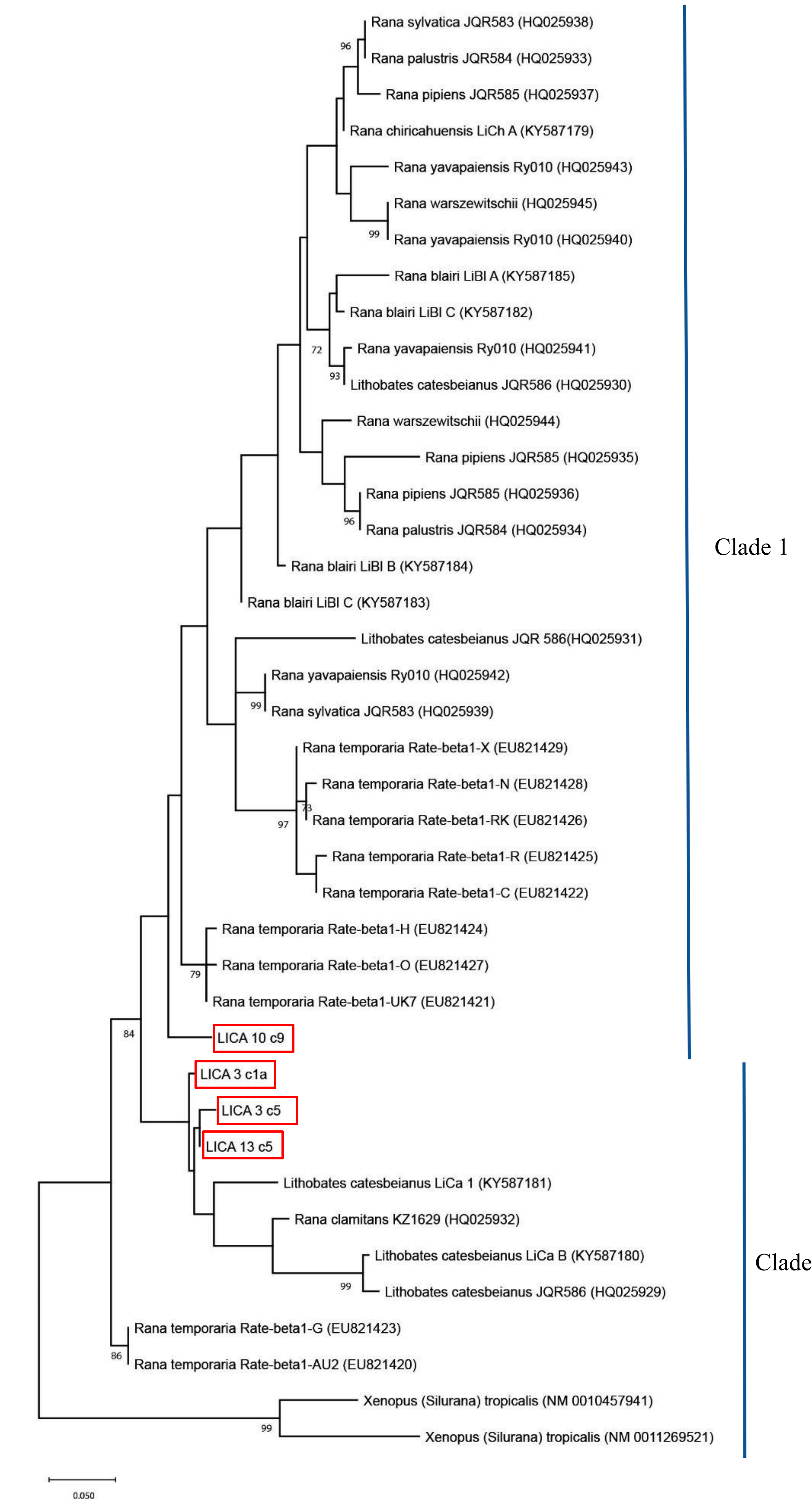
## Conclusions

- Our data support our hypothesis that bullfrogs have moderate allelic diversity and this may have attributed to their successful colonization of Humboldt county
- Positive selection was detected on putative binding sites of MHC in a larger dataset including our alleles
- Some allelic lineages have likely been maintained since before speciation of *Lithobates*

## Acknowledgements

- We thank:
- James Bettaso (U.S. Forest Service) for providing bullfrog tissues
  - Anna Savage (University of Central Florida) for providing PCR protocols
  - The Cal Poly Humboldt Department of Biological Sciences for funding the project

## Phylogenetic reconstruction of MHC alleles reveals presence of two distinct lineages



## References

Barribeau, S.M., et al. (2008) Major Histocompatibility Complex based resistance to a common bacterial pathogen of amphibians. *PLoS ONE* 3: e2692

Batallic, A., et al. (2015) Susceptibility of amphibians to chytridiomycosis is associated with MHC class II conformation. *The Royal Society Publishing* 282: 20143127

Hammond, S.A., et al. (2017) The North American bullfrog draft genome provides insight into hormonal regulation of long noncoding RNA. *Nature Communications* 8: 1433

Hauswaldt, J.S., et al. (2007) Molecular characterization of MHC class II in a nonmodel anuran species, the fire-bellied toad *Bombina orientalis*. *Immunogenetics* 59: 479-491

Kosch, T.A., et al. (2016) Major histocompatibility complex selection dynamics in pathogen-infected tungara frog (*Physalaemus pustulosus*) populations. *Biology Letters* 12: 20160345

Laufer, G., et al. (2018) Current status of American bullfrog, *Lithobates catesbeianus*, invasion in Uruguay and exploration of chytrid infection. *Biological Invasions* 20: 285-291

Mulder, K., et al. (2017) Evolutionary dynamics of an expressed MHC class IIβ locus in the Ramidae (Anura) uncovered by genome walking and high-throughput amplicon sequencing. *Developmental and Comparative Immunology* 76: 177-188

Savage, A.E., and Zamudio, K.R. (2011) MHC genotypes associate with resistance to a frog-killing fungus. *Proc Natl Acad Sci U. S. A.* 108: 16705-16710

Teacher, A.G.F., et al. (2009) Evidence for directional selection at a novel Major Histocompatibility Class I marker in wild Common frogs (*Rana temporaria*) exposed to a viral pathogen (ranavirus). *PLoS ONE* 4: e4616