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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

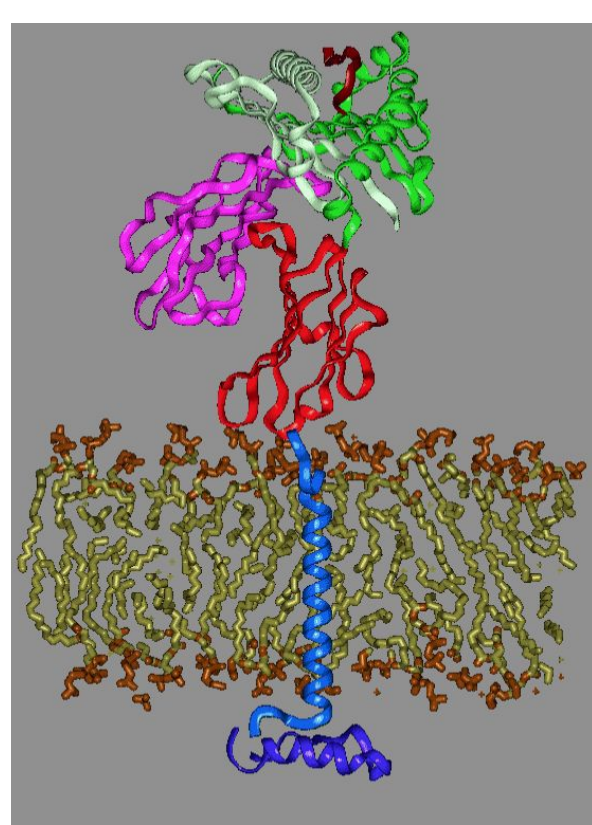
- American Bullfrogs (*Lithobates catesbeianus*) are native to the East Coast of the U.S.
- These frogs are highly invasive and have successfully colonized different habitats all over the world
- They have a number of negative effects on the ecosystem they colonize:
 - Introduce new diseases
 - Cause 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)

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)



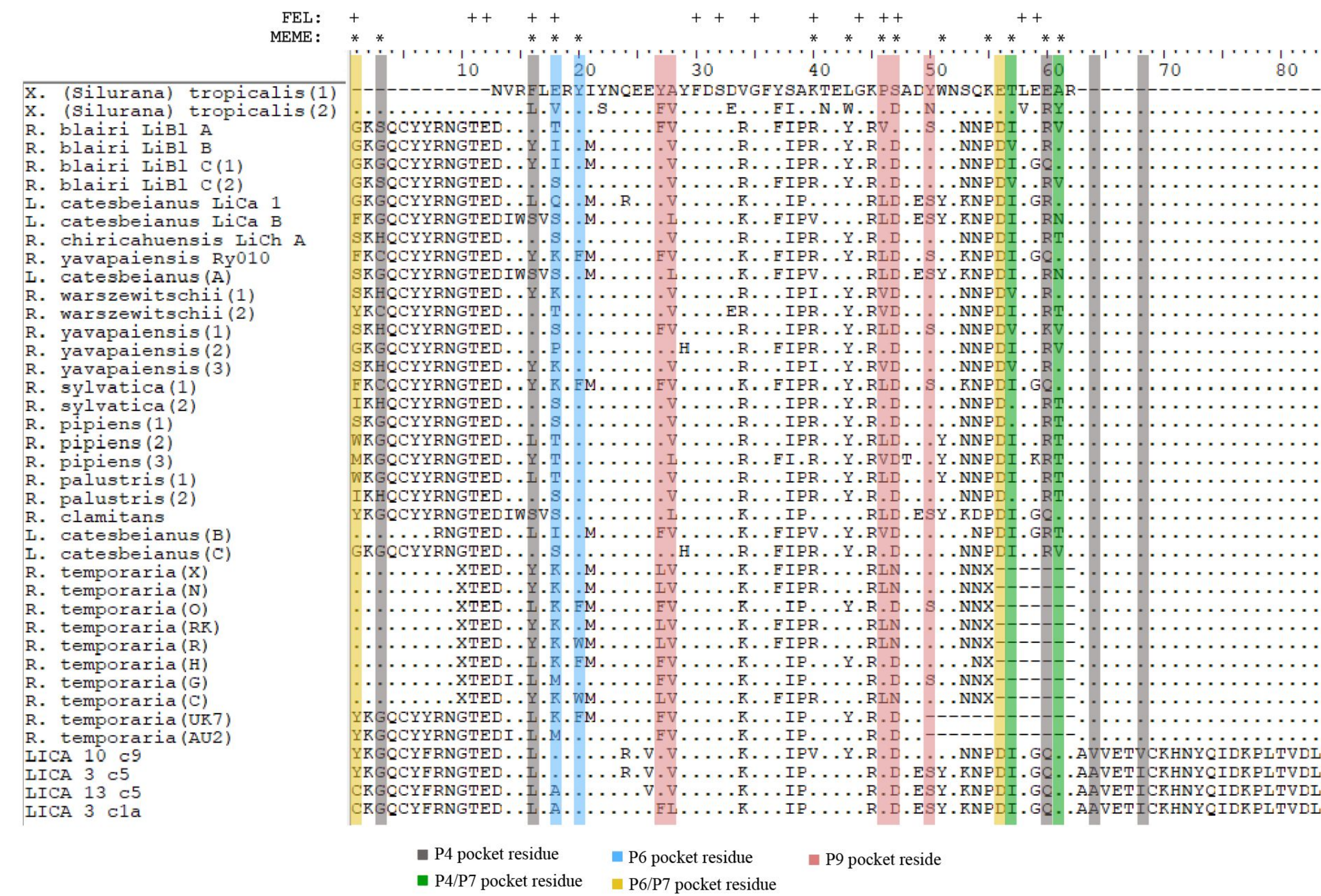
- Having different alleles can influence disease susceptibility to viral, bacterial, and fungal infections in frogs (e.g., Savage and Zamudio 2011, Barribeau et al. 2008, Teacher et al. 2009)

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>)

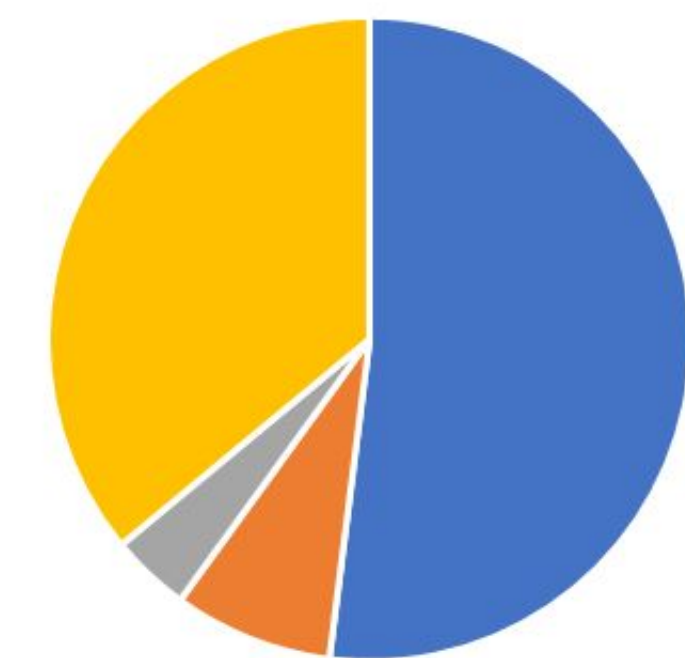
Methods

- 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



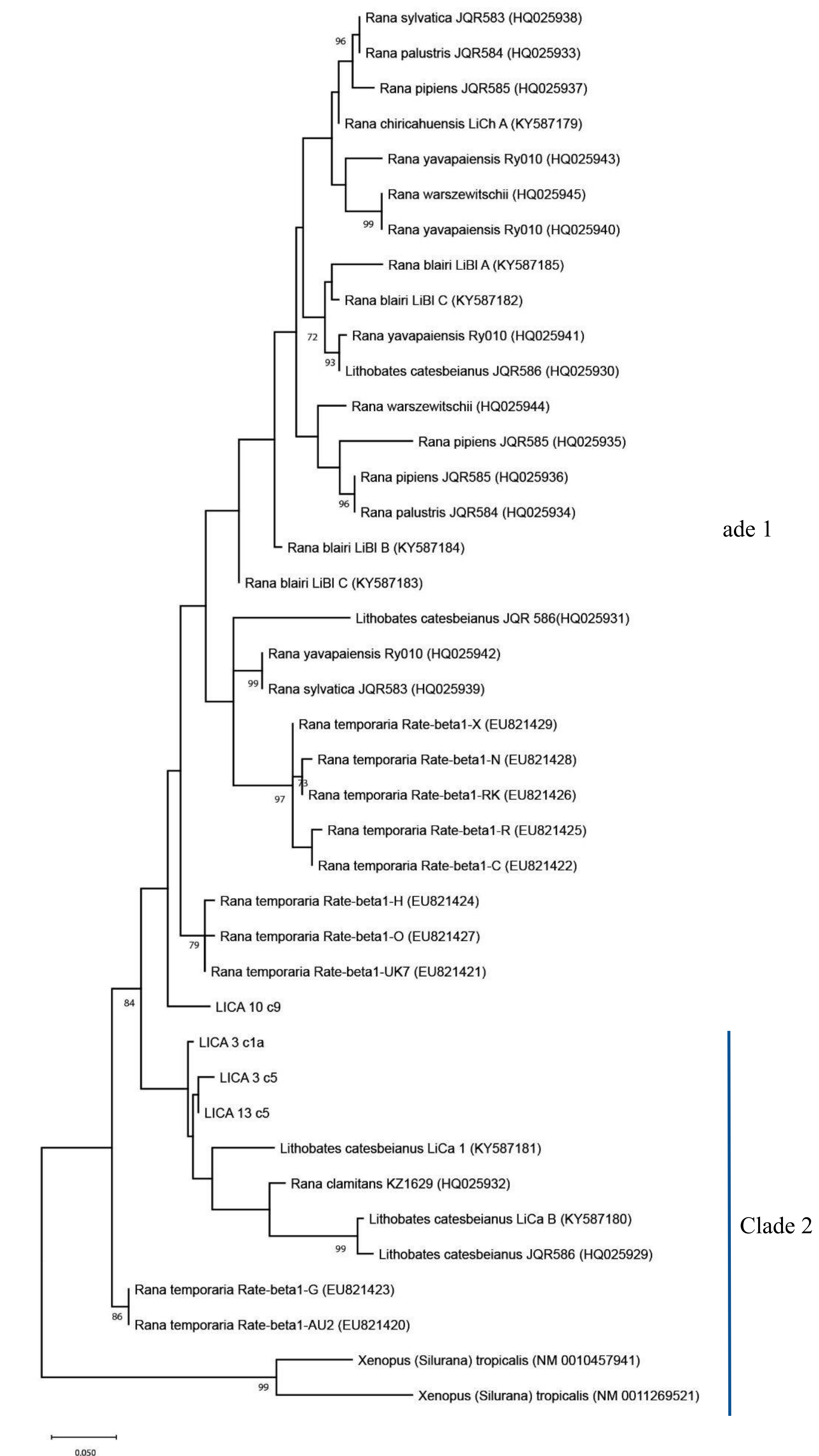
Conclusions

- 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

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Phylogenetic reconstruction of MHC alleles reveals presence of two distinct lineages



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