

## Background

The amphibian olfactory system typically functions for aerial and aquatic olfaction<sup>1,2</sup>. In salamanders, the peripheral olfactory system is comprised of two major parts: the main olfactory cavity (MOC) and the vomeronasal organ (VNO). Sensory cells that detect chemical signals are located in the lining of these cavities, and each animal can express hundreds of different receptor proteins that bind to different odor molecules.

To begin to understand how variable the olfactory receptors are in a local salamander, we used multiple methods to isolate olfactory receptor DNA sequences from the California Slender Salamander (*Batrachoseps attenuatus*), a direct-developing species (no larval stage) that ranges from coastal Northern California to southern Oregon<sup>5</sup>. Plethodontid salamanders are small salamanders that rely on olfaction for a variety of social behaviors, such as courtship and territory recognition<sup>3,4</sup>.



Figure 1. Photograph of an adult California slender salamander.

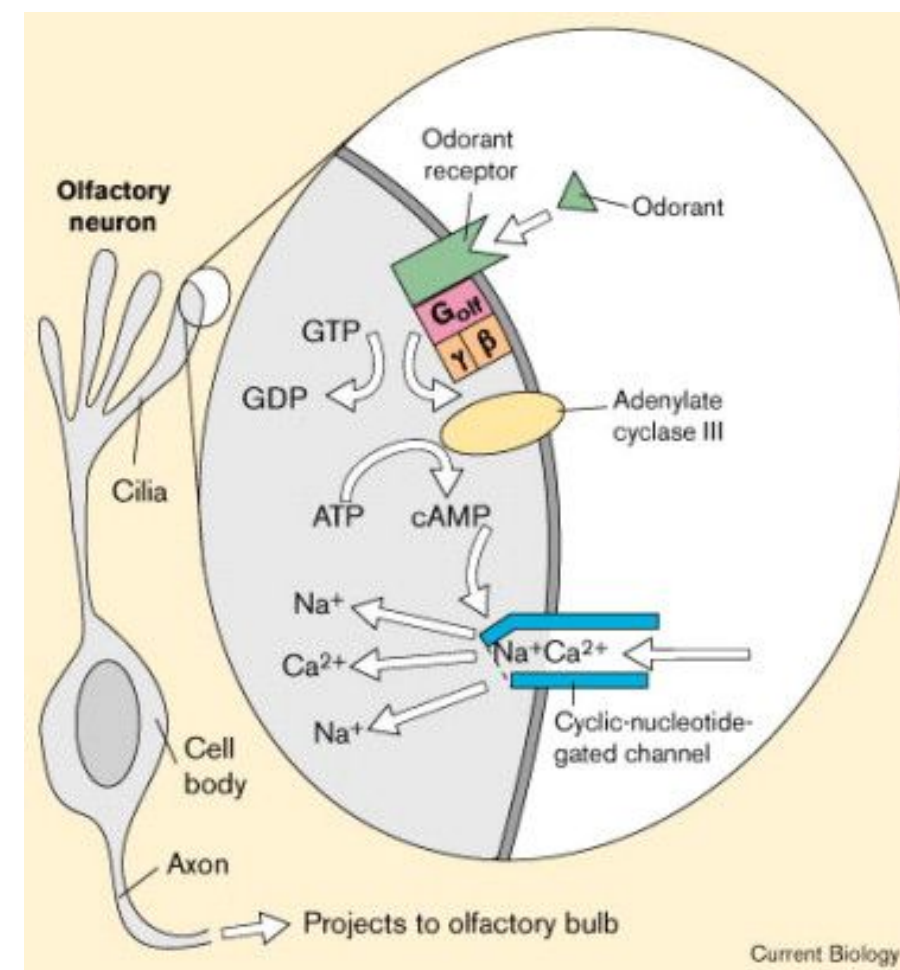


Figure 2. Schematic of the structure of an olfactory receptor (OR) protein<sup>5</sup>.

## Phylogenetic analysis reveals the presence of at least one family of receptor genes in slender salamanders

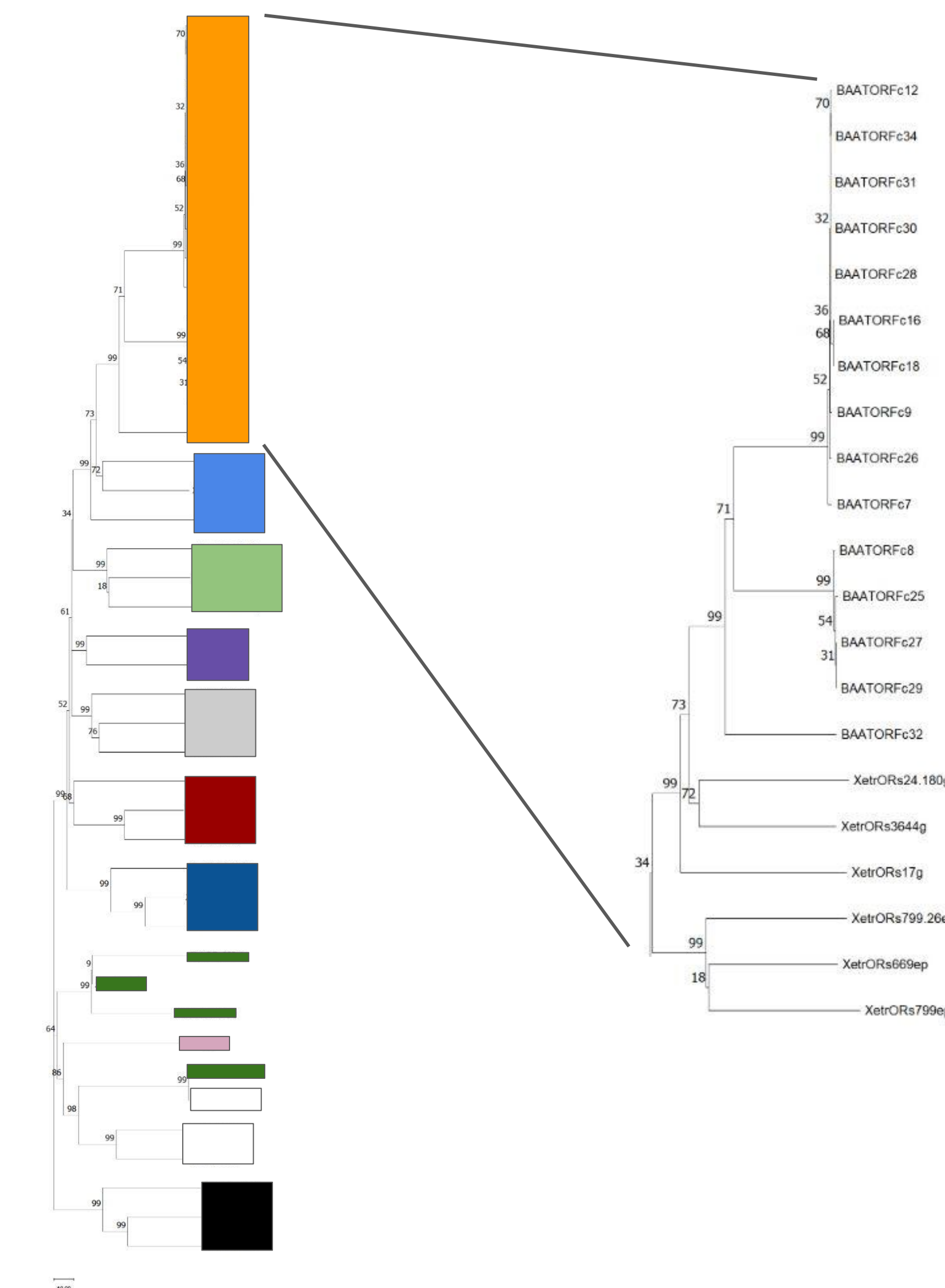


Figure 3. Phylogenetic reconstruction of major families (alpha-lambda) of olfactory receptors combined with the ones we isolated. Colors represent major OR families: orange = ones from this study, grey = alpha, dark blue = beta, cornflower blue = gamma, dark red = delta, light green = epsilon, purple = zeta, dark green = eta, white = theta, pink = kappa, black = lambda. Inset shows subtree of gamma family sequences. Scale bars = substitutions per site.

## OR genes are highly variable

Table 1. Matrix of pairwise DNA sequence differences among ORs isolated in this study. Values indicate the number of differences between two sequences of receptors. Maximum number of differences was 519.

	BAATORFc12	BAATORFc16	BAATORFc25	BAATORFc26	BAATORFc27	BAATORFc32	BAATORFc7	BAATORFc8	BAATORFc9	XetrORs17g	XetrORs24.180g	XetrORs3644g
BAATORFc12												
BAATORFc16	1.00											
BAATORFc25	64.00	65.00										
BAATORFc26	1.00	2.00	65.00									
BAATORFc27	63.00	64.00	1.00	64.00								
BAATORFc32	69.00	70.00	73.00	70.00	72.00							
BAATORFc7	2.00	3.00	66.00	3.00	65.00	70.00						
BAATORFc8	63.00	64.00	2.00	64.00	1.00	72.00	65.00					
BAATORFc9	1.00	2.00	65.00	2.00	64.00	69.00	3.00	64.00				
XetrORs17g	100.00	100.00	110.00	100.00	109.00	107.00	100.00	108.00	100.00			
XetrORs24.180g	99.00	100.00	101.00	98.00	100.00	90.00	99.00	100.00	99.00	109.00		
XetrORs3644g	92.00	93.00	95.00	92.00	94.00	86.00	92.00	95.00	92.00	98.00	92.00	

## Conclusions

- This first characterization revealed 3 different subtypes of receptors, all belonging to the gamma family of vertebrate ORs<sup>7</sup>. Although all our sequences grouped with gamma family, they were variable (differences between them ranged from 1-73 nucleotides).
- These isolated gene fragments are likely to encode functional receptors because they have predicted transmembrane domains, conserved cysteines and other residues necessary for protein function

### Future directions

- Future studies will focus on using next-generation sequencing techniques to more fully characterize the full repertoire of olfactory receptor subtypes.
- We also plan to investigate whether there are differences in expression between males and females.

## Methods

### Sample Collection

- Animal was collected in Humboldt County, CA (CA Collecting Permit S-200260005-20362-001).

### RNA extraction and cDNA synthesis

- Total RNA was extracted from olfactory tissue and reverse-transcribed into cDNA using standard protocols.

### PCR, cloning and sequencing

- Approximately 500 bp of receptor genes were amplified using primers designed by Zhou et al.<sup>6</sup>
- PCR products were cloned using the pGEM-Teasy vector system (Promega Corporation) and 15 different purified plasmids were sequenced at Eurofins Genomics (Louisville, KY).

### Alignment and distance calculations

- DNA and amino acid alignments was constructed using the MUSCLE program implemented in MEGA.
- Distance matrix of pairwise DNA differences was calculated in MEGA.

### Phylogenetic analysis

- Representatives of major OR families from clawed frogs were taken from GenBank and aligned with ours.
- This alignment was used to make a phylogenetic tree using the neighbor-joining method in MEGA (100 bootstrap reps).

## Salamander ORs have conserved residues necessary for their function as chemoreceptors

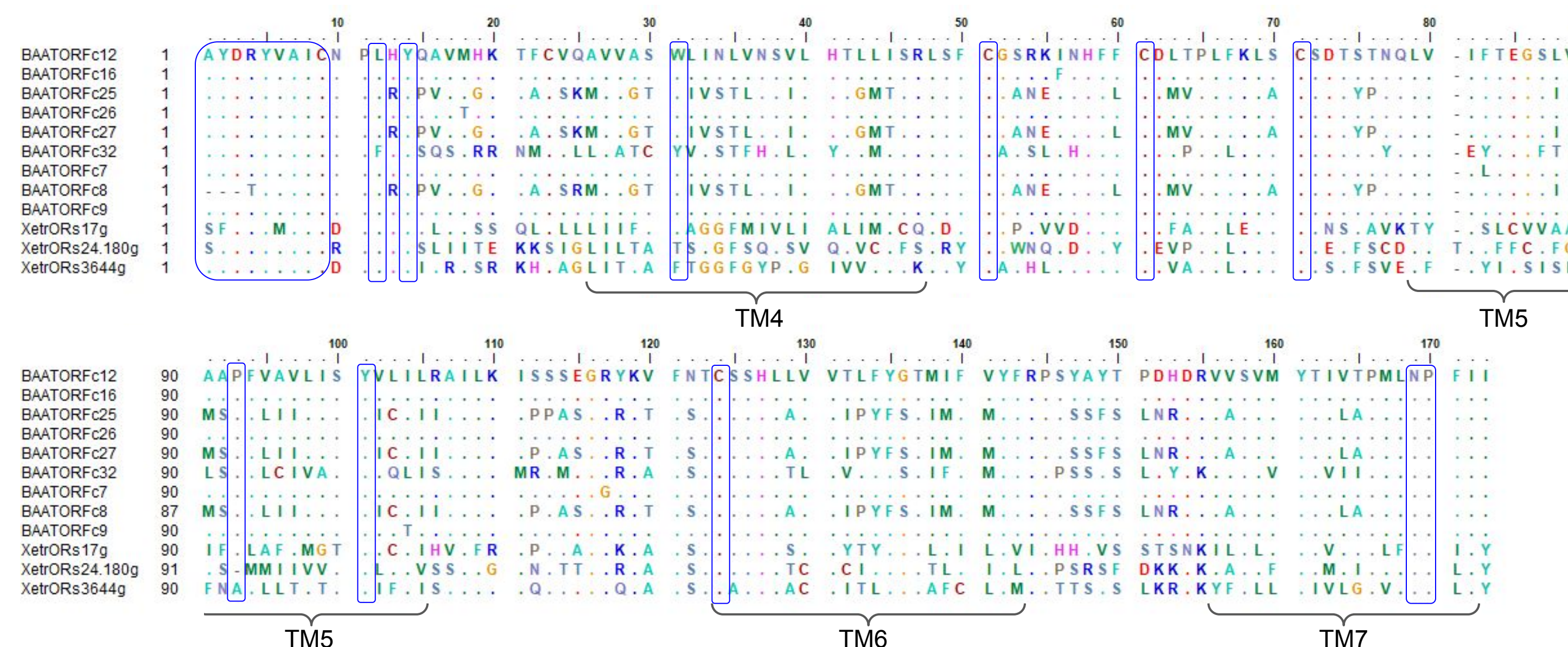


Figure 4. Amino acid alignment of slender salamander ORs (BAATORs) with a subset of ORs from clawed frogs (Xetr). Regions and residues important for receptor function are noted: TM = putative transmembrane domain and blue boxed areas are conserved residues found in most olfactory receptors<sup>8</sup>.

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## References

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