



USING microCT DATA TO STUDY THE EVOLUTION OF OPISTHOGLYPHOUS DENTITION IN COLUBRID SNAKES

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INTRODUCTION

Many extant snakes (Order Squamata, Suborder Serpentes) possess fangs, specialized teeth on the maxillary bone that serve the purpose of envenomating prey.^{1,2} All venomous snakes belong to the superfamily Colubroidea, but different fang types were once thought to have evolved independently within this group.^{2,3} Family Elapidae is comprised of snakes with proteroglyphous fangs, while Viperidae contains snakes with solenoglyphous fangs.⁴ Snakes in the family Colubridae were traditionally considered aglyphous and non-venomous.⁵ However, many Colubrids actually possess opisthoglyphous dentition, in which the fangs are located on the posterior of the maxilla and are grooved rather than hollow, but can still envenomate prey.^{6,7} Recent evidence has shown that venom glands and fangs are likely Colubroid synapomorphies.^{8,9}

Examination of museum specimens of 81 species of snakes helped to answer the following questions regarding opisthoglyphous dentition:

1. How are various dental characteristics related across these species?
2. What was the most likely state of the posterior teeth in the Colubrid ancestor?
3. Is there phylogenetic signal in the distribution of rear fangs across Colubridae?
4. What is the mode of evolution of rear fangs across Colubridae?
5. How much variation is present in specific groove dimensions of the rear fangs?



METHODS

I examined specimens from the University of Michigan Museum of Zoology of species that were contained in the phylogenetic estimate provided by Pyron et al.¹⁰ For each specimen, I counted teeth on each of the four tooth-bearing bones (maxillary, palatine, pterygoid, dentary) and recorded whether the specimen had enlarged anterior maxillary teeth, enlarged posterior maxillary teeth, and / or grooved posterior maxillary teeth. A subset of specimens was taken for μ CT scanning (SoD mCT core, funded in part by NIH/NCRRI S10RR026475-01). Specimens were placed in a 34 mm diameter tube and scanned using a microCT system (μ CT100 Scanco Medical, Bassersdorf, Switzerland). Scan settings were: voxel size 12 μ m, 70 kVp, 114 μ A, 0.5 mm AL filter, 750 projections and integration time 750 ms. Data was returned in the form of image stacks. Using Avizo 9.0.2 software, I created surface and volume renditions from the μ CT images.

1. I analyzed intra-family variability in tooth counts per bone, as well as differences in the number of maxillary teeth for species that possessed a rear fang as compared to those that did not. I also investigated the co-occurrence of different dental characters across species. All analyses were performed in R version 3.3.2.¹¹

2. To estimate ancestral states, I used a maximum likelihood approach under a Markov model of discrete character evolution, using the 'asr' function in the 'diversitree' package in R.¹² The posterior probabilities for each character state at each internal node were determined. I also performed a parsimony reconstruction of the tree, which uses an algorithm to minimize the number of character state changes, using functions adapted from those in the 'phangorn' package.^{13,14}

3. As an estimate of phylogenetic signal, I computed the maximum parsimony estimate for the distribution of rear fangs across the phylogeny using functions adapted from the 'phangorn' package.^{13,14} Then I randomly assigned character states to the tips of the tree based on their observed frequency. I computed the number of parsimony changes from the random distribution for each replicate. I performed this simulation 10,000 times to generate a null distribution, and compared this to the observed value of inferred parsimony changes for rear fangs across the phylogeny.

4. I created a likelihood function for single discrete trait evolution for the subtree containing species analyzed here and the distribution of rear fangs, using the function 'make.mk2' in diversitree.¹² Using the 'find.mle' function, I ran a maximum likelihood analysis to obtain the instantaneous rate matrix for an aglyphous state to rear fangs (q_{01}) and vice versa (q_{10}) that maximized the probability of the observed character distribution. I estimated two models: one in which transition rates were constrained to be equal, and one in which the transition rate from no fangs to rear fangs was allowed to differ from that of the reverse, and performed a log-likelihood ratio test to compare the models.

5. In Avizo, I segmented individual grooved teeth from the maxillary bone of the surface rendition. I took three measurements of groove length and tooth length per grooved tooth. I also measured groove width, tooth width, groove depth and tooth depth at 10 equidistant points along the surface of the tooth. I averaged these measurements to get an estimate of average groove dimensions for each grooved tooth per specimen.

RELATIONSHIPS BETWEEN DENTAL CHARACTERS

40 of 81 species examined possessed rear fangs. All rear fanged species were in the family Colubridae. Within Colubridae, 30 of 44 species in subfamily Dipsadinae had rear fangs, while 10 of 21 in Colubrinae had rear fangs. Colubrinae had significantly more teeth on the maxillary bone than did Dipsadinae ($t = -2.3849$, p -value = 0.0244). Colubrinae also had more teeth on the palatine bone than Dipsadinae ($t = -2.5475$, p -value = 0.01448). The relationship between the presence of a fang and number of teeth on the maxilla was significant ($t = -2.2254$, p -value = 0.02939); snakes that possess a rear fang have significantly fewer teeth total on the maxillary bone than those that do not have a fang. Distributions of grooved posterior teeth and enlarged posterior teeth were significantly related, suggesting their distributions are not independent ($\chi^2 = 21.302$, p -value < 0.0001).

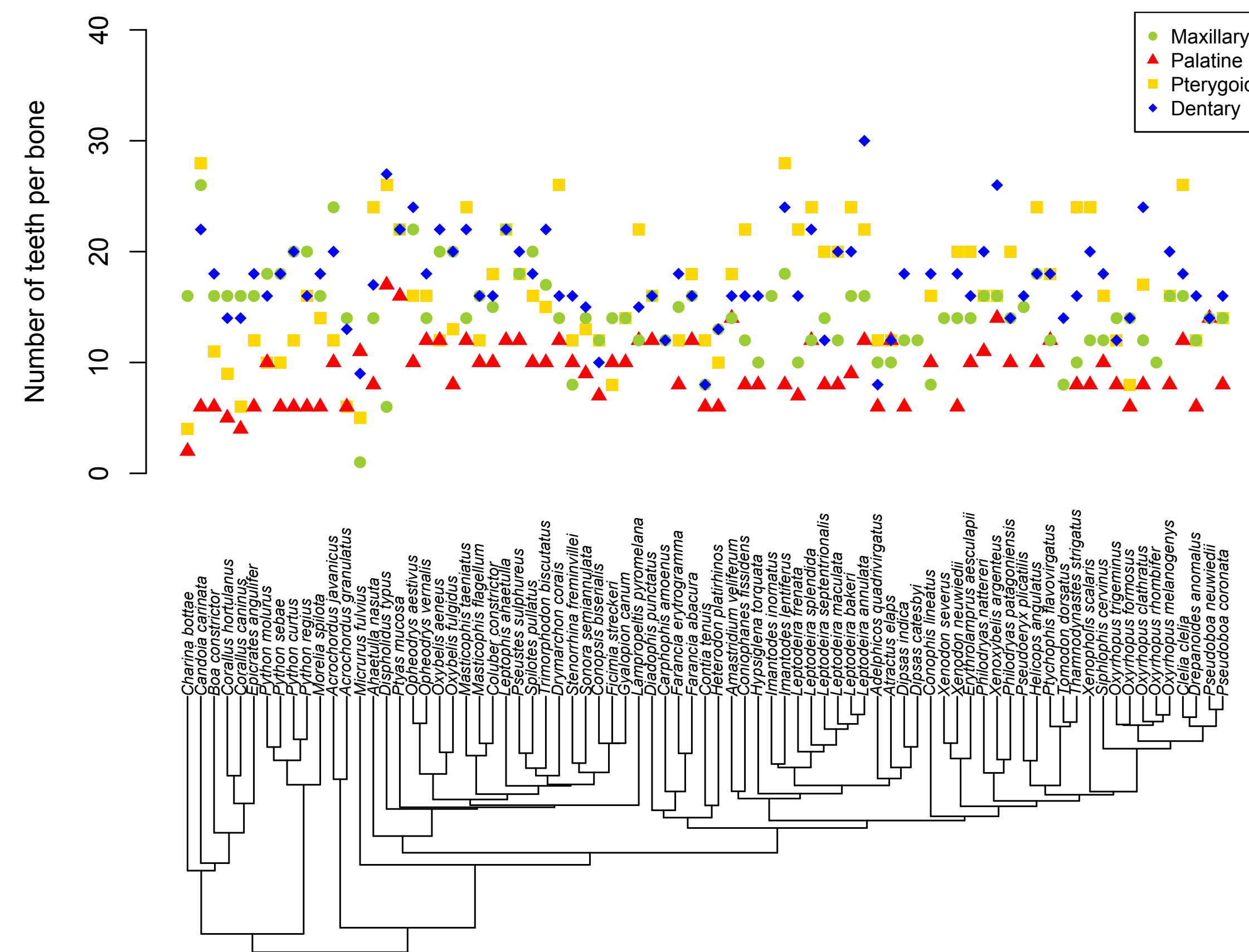


Fig. 1: tooth counts for each tooth bearing bone of species considered here across a subtree created from the phylogenetic estimate provided by Pyron et al., 2013.¹⁰

ANCESTRAL STATE RECONSTRUCTION

Character state probabilities at the Colubroid common ancestor node revealed strong evidence for a rear fanged ancestral state (probability of no rear fangs: 0.000, probability of rear fangs: 1.000). Evidence also supports a rear fanged common ancestor of the family Colubridae (probability of no rear fangs: 0.000, probability of rear fangs: 1.000). Probability estimates for all internal nodes are displayed in figure 2. Parsimony reconstruction suggests a rear fanged common ancestor for family Colubridae, with several repeated losses and subsequent gains of rear fangs throughout the family history. Concordant estimates from two distinct methods here provide strong evidence for a rear fanged Colubrid ancestor.

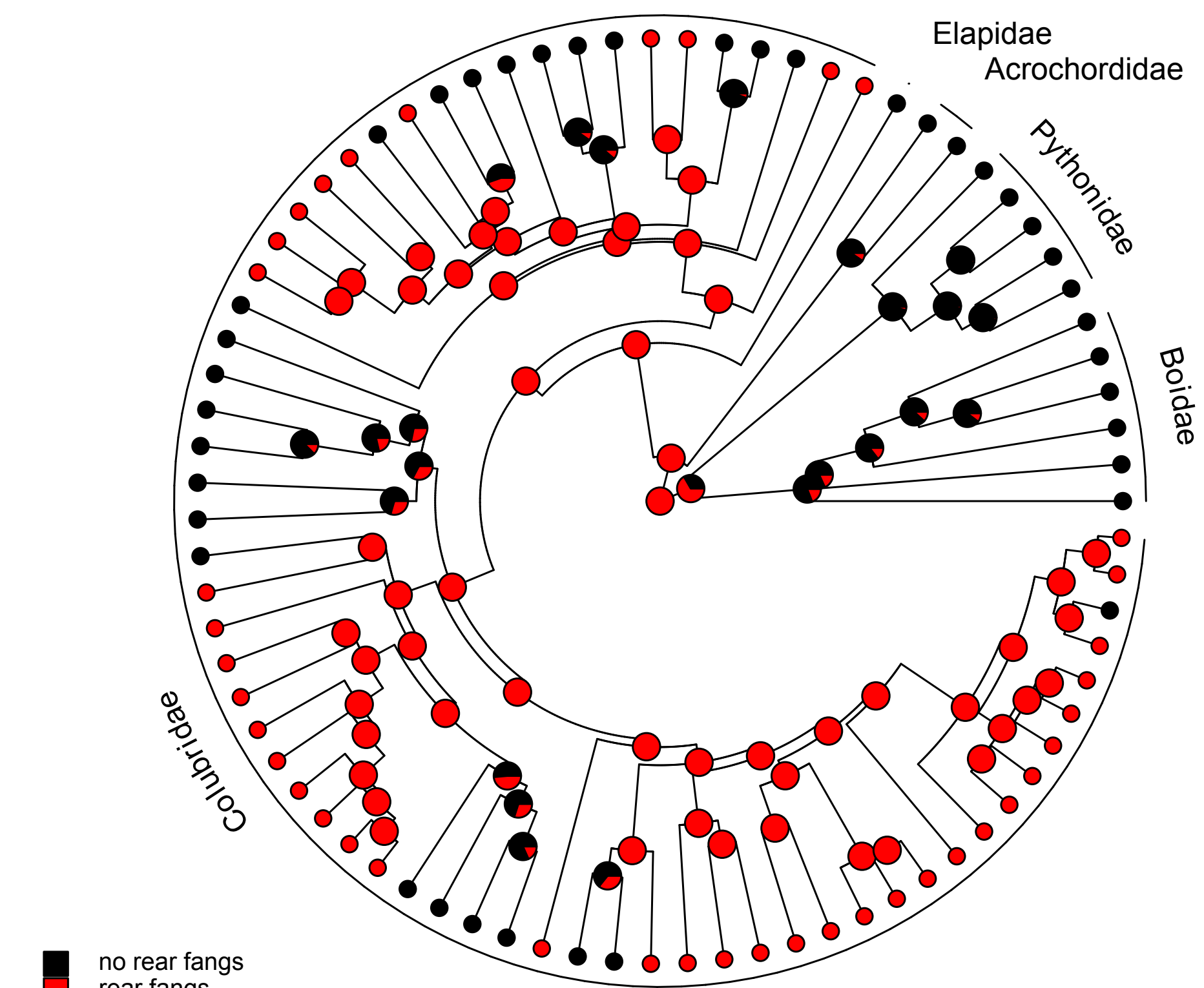


Fig. 2: results of likelihood estimation of ancestral states. Colors in the pie charts of internal nodes represent their distribution of posterior probability of belonging to one of the two character states (lacking rear fangs, black, or possessing rear fangs, red). The identity of terminal nodes, i.e. extant species, was considered as a fixed state.

PHYLOGENETIC SIGNAL

The observed value of maximum parsimony changes for the presence or absence of rear fangs across the phylogeny was 10. In 10,000 simulations, I recorded 0 instances where the calculated parsimony changes for the randomly distributed character states were equal to or less than the observed value, evidence of strong phylogenetic signal of rear fangs (p -value < 0.0001).

MODEL OF CHARACTER EVOLUTION

I fitted two different Markov models of discrete character evolution: one in which the transition rates q_{01} and q_{10} were constrained to be equal, and one in which they were allowed to vary. The first model gave a transition rate $q_{01} = q_{10} = 0.00629$, with a likelihood = -39.03646. For the second model, I obtained transition rates $q_{01} = 0.00650$ and $q_{10} = 0.00586$ with a likelihood = -39.03068. The log likelihood comparison of the constrained model / non-constrained model gave a value of 1.000148, p -value = 1, $df = 1$, suggesting rates q_{01} and q_{10} do not differ significantly.

VARIATION IN GROOVE DIMENSIONS

Of 36 unique species of which specimens were μ CT scanned, 12 were found to possess significant grooves on the rear maxillary teeth. I found a large amount of variability within the category of posterior grooved teeth, i.e. rear fangs. I fitted a linear model to the data of groove width vs. groove depth. The resulting model returned a significant, negative relationship between groove width and depth ($F = 7.708$, $r^2 = .435$, p -value = 0.01957).

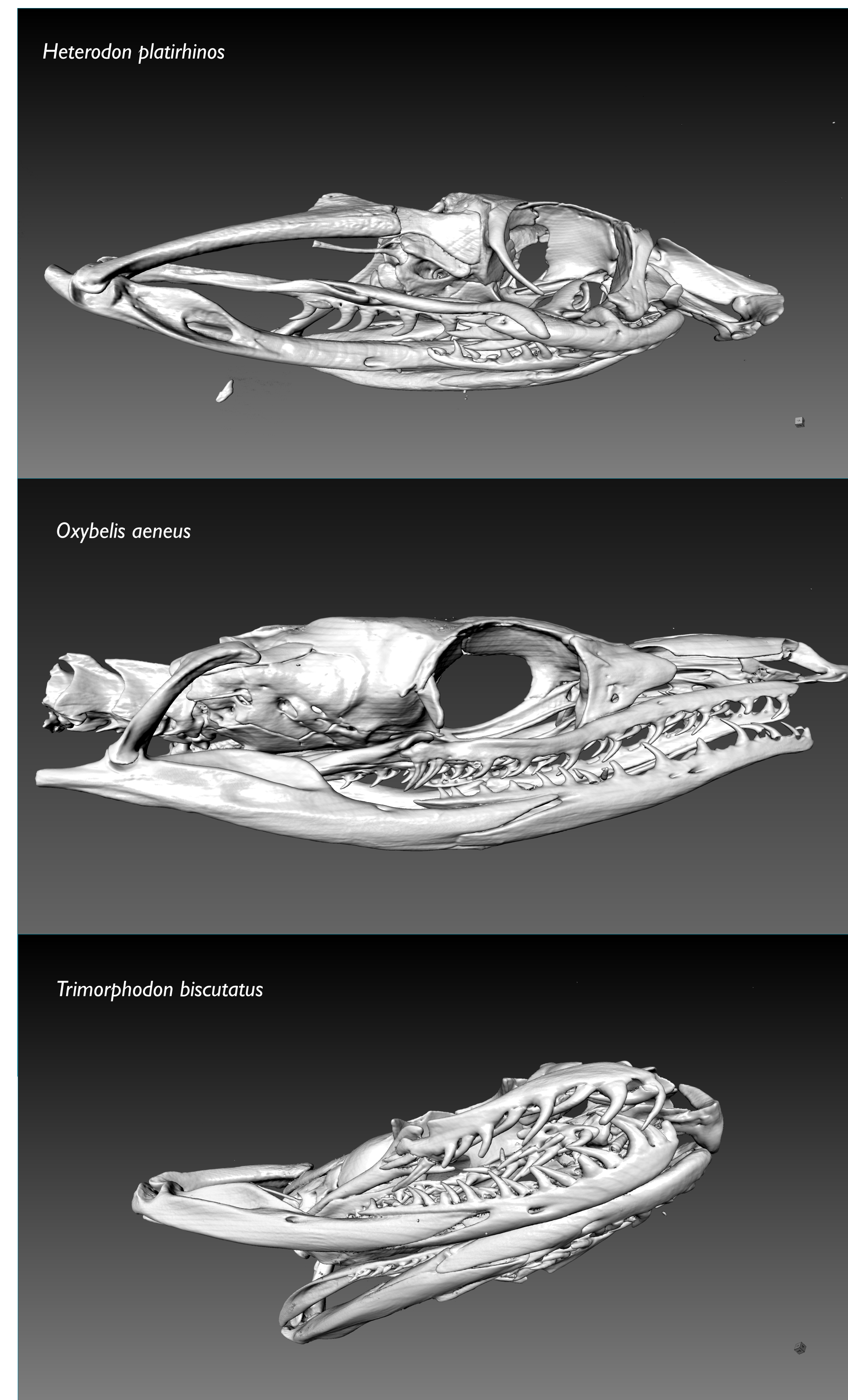


Fig. 3: example volume renditions highlighting diversity of rear fang morphology. Top: *Heterodon platirhinos* exhibits enlarged but ungrooved posterior maxillary teeth. Middle: *Oxybelis aeneus* possesses multiple enlarged and grooved posterior teeth with grooving on the labial surface of the tooth. Bottom: *Trimorphodon biscutatus* with recurved, enlarged maxillary fangs with grooving on the anterior surface of the tooth.

CONCLUSIONS

- ◆ Analysis of μ CT data proves a promising method for uncovering details about the evolution of this trait. μ CT reconstructions made accurate tooth counts possible. Some specimens are preserved in such a way that certain tooth-bearing bones obscure the view of others, and accurate counts cannot be made without modification of the specimen, which can be destructive. μ CT scanning of specimens bypasses this obstacle. Moreover, μ CT data allows for analysis of surficial aspects of grooved teeth that would be impossible to quantify with microscopy alone.
- ◆ Tooth counts within Colubridae were highly variable, with Colubrinae possessing more teeth on both the maxillary bone and palatine bone as compared to Dipsadinae. The negative relationship between presence of fangs and number of maxillary teeth may suggest an evolutionary trade-off, but this relationship requires more investigation in Colubrids. The significant relationship between enlargement and grooving on the posterior maxillary teeth suggests that these traits frequently, but not always, occur together. The interplay between these two characteristics of the posterior maxillary teeth proves interesting and is worth further study.
- ◆ Results from two methods of ancestral state reconstruction provide strong evidence that the common Colubrid ancestor possessed rear fangs. The distribution of rear fangs seen here is best explained by evolution of rear fangs at the base of the Colubrid radiation, followed by subsequent losses in some lineages.
- ◆ Comparisons between the maximum parsimony estimate for the observed distribution of rear fangs compared that of the simulated, random distribution of rear fangs showed strong phylogenetic signal, indicating a conserved pattern of character evolution of this trait.
- ◆ The model of character evolution that best fit my data gave asymmetric rates for losses and gains of rear fangs. The rate estimate gave a slightly higher rate of evolution for the gain of rear fangs as compared to the loss at the species level. However, the log-likelihood comparison of the constrained model and non-constrained model was non-significant, suggesting that the rates of gain and loss of this trait are fairly equivalent. Repeated loss of the grooving on the rear teeth could suggest this trait is particularly evolutionarily labile.
- ◆ μ CT data analysis revealed that relative groove width is negatively correlated with relative groove depth. Species with proportionately deeper grooves tend to have proportionately narrower grooves.
- ◆ By obtaining μ CT images and subsequently analyzing surface and volume data, I was able to quantify aspects of the degree of grooving in rear fanged snakes. With microscopy alone, much of the variation in this trait can be lost. Previously, studies have differentiated teeth into broad categories, and have described only the relative groove length for grooved posterior teeth.¹⁵ μ CT scanning and analysis of imagery proves an effective method to examine and quantify other aspects of this highly variable trait, such as groove depth and width. More complete sampling with μ CT could allow one to test for phylogenetic signal and mode of evolution of specific groove quantities.

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