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Michael G. Sovic  
*The Ohio State University*

Kody F. Kuehnl  
*Franklin University, kody.kuehnl@franklin.edu*

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# Mitochondrial DNA Suggests Stonerollers (*Campostoma anomalum*) From Upper Shavers Fork Have Strong Genetic Similarities With The Recently Identified New River Form

Michael G. Sovic<sup>1</sup> and Kody F. Kuehn<sup>1,2</sup>

<sup>1</sup> Dept of Evolution, Ecology, and Organismal Biology, The Ohio State University

<sup>2</sup> Dept of Social and Natural Sciences, Franklin University

## Background

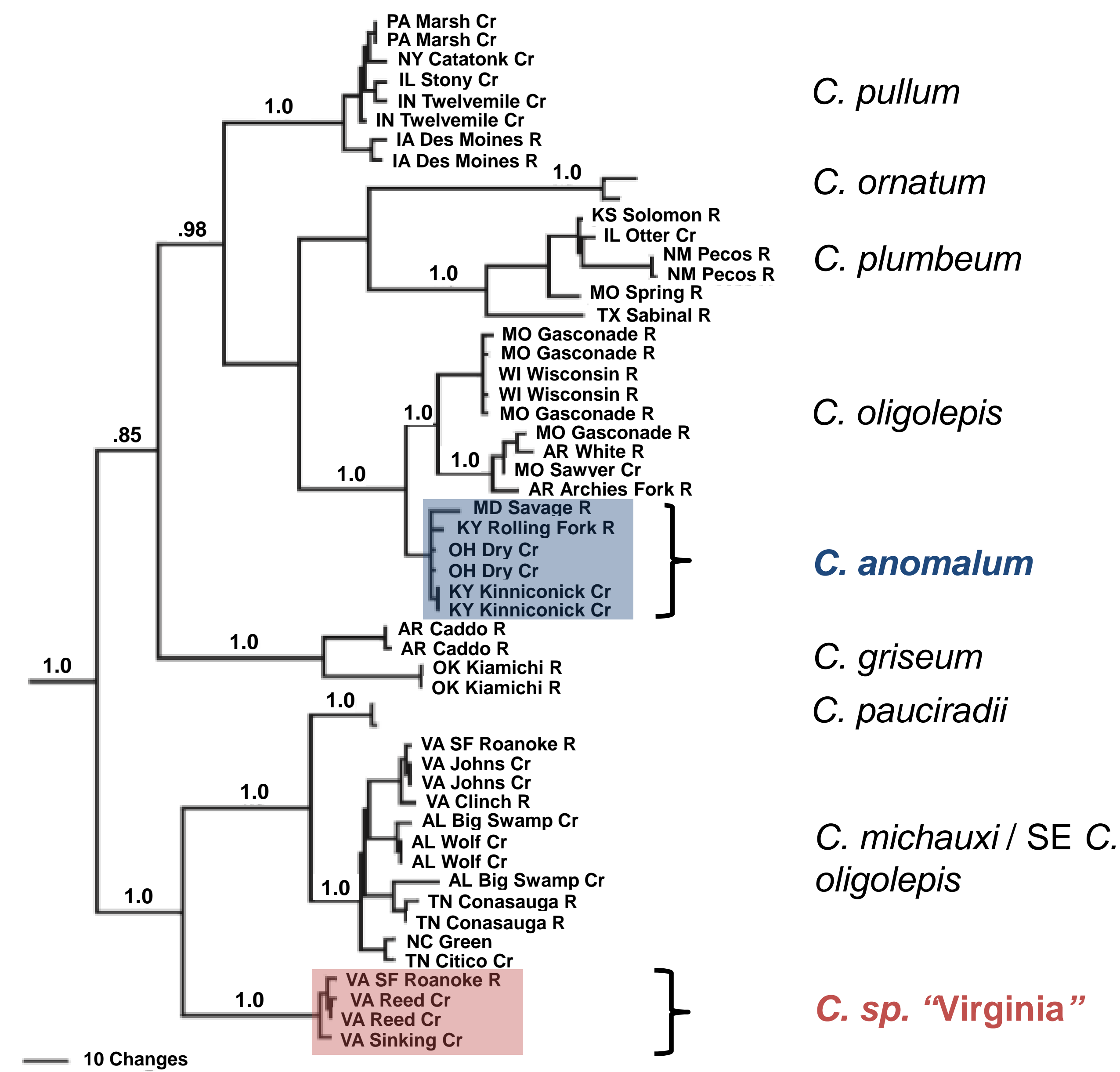
- Mitochondrial DNA sequence data can offer insights into the occurrence and patterns of cryptic biodiversity that may not be evident with other types of data.
- A recent analysis of Cytochrome b sequence variation revealed a number of previously-unrecognized lineages within *Campostoma* of the central and eastern United States (Blum et al. 2008).
- Two of the identified lineages occur in West Virginia: *C. anomalum*, which occurs throughout waters of the Ohio and Potomac River basins, and a previously undescribed form from the New and Roanoke River drainages (Fig. 1).
- Blum et al. (2008) tentatively referred to the New/Roanoke River form as *C. sp.* "Virginia", and recommended that it be given formal taxonomic recognition.
- In this study, we generated cytochrome b DNA sequence data for stonerollers from additional localities (Shavers Fork of the Cheat River, and the Cacapon River drainage) to further examine the geographic distribution of the two distinct *Campostoma* lineages that occur in West Virginia.

## Methods

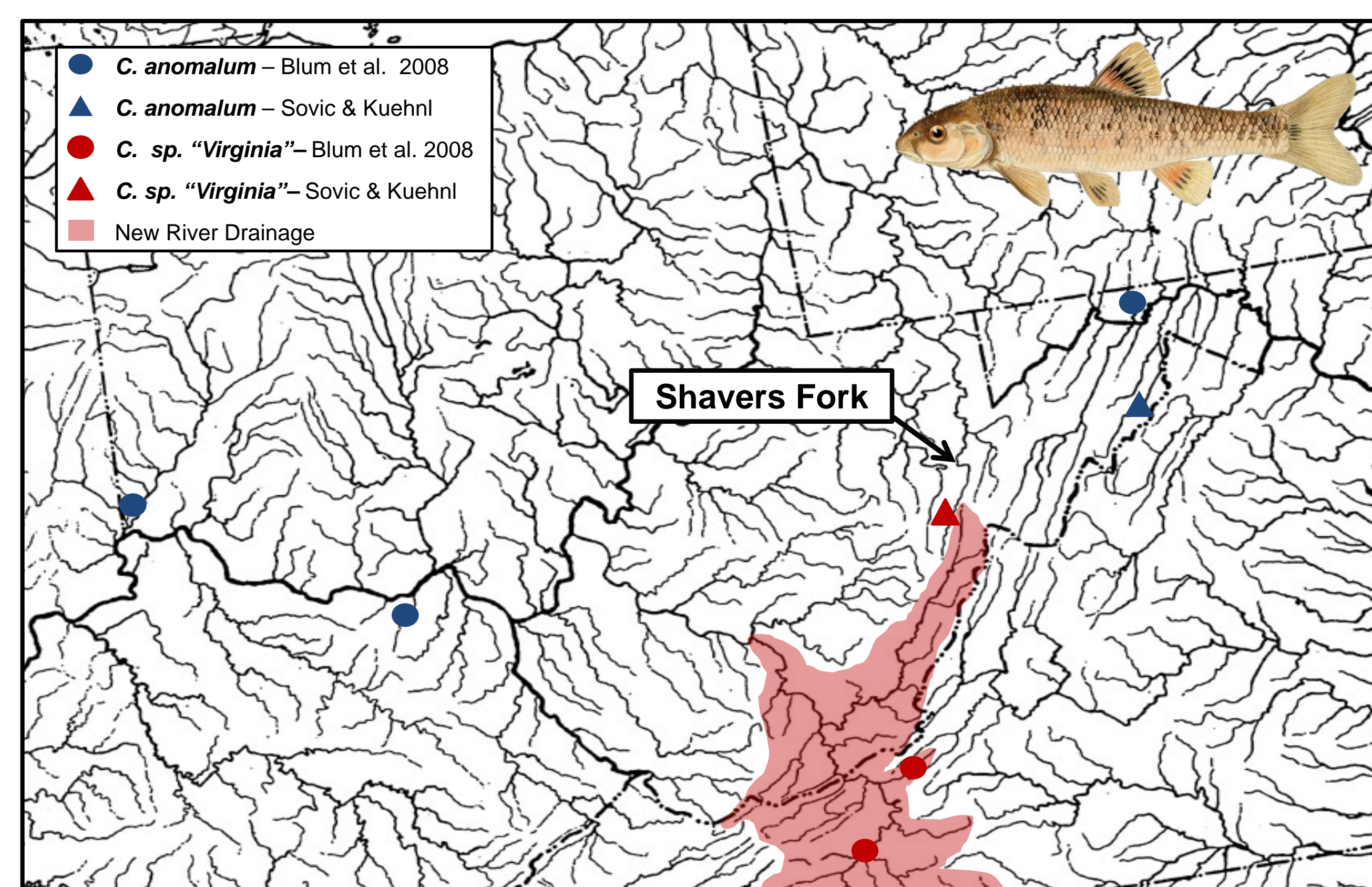
- Stonerollers were sampled in upper Shavers Fork (multiple locations upstream of the High Falls on Shavers Fork; N=5) and Waits Run (Cacapon River; N=2). Fin clips were obtained and stored in 70% ethanol.
- DNA was extracted and the cytochrome b gene was sequenced for each individual.
- Additional cytochrome b sequences representing the *C. anomalum* clade and the *C. sp.* "Virginia" clade, as defined by Blum et al. (2008), were obtained from Genbank.
- Phylogenetic analysis was performed using TNT v. 1.1. (Goloboff, Nixon, and Farris 2000) under the parsimony criterion (MP). Nodal support was determined using 10,000 jackknife replicates.
- The dataset was also analyzed under the maximum likelihood criterion (ML) using RAxML-VI-HPC (Stamatakis 2006) and was subjected to 1000 rounds of bootstrap resampling to assess support for clades.
- The outgroup taxon, *C. ornatum*, was chosen based on the phylogeny of Blum et al. (2008).

## Acknowledgements

Many thanks to Erin Sovic, David Sovic, and Adam Andrews for assistance during sample collection, and to the students in EEOB 713 (2010) for their efforts in generating preliminary data for this study.



**Figure 1.** Major lineages within *Campostoma* as identified and presented by Blum et al. (2008). Highlighted lineages are those that occur in West Virginia. Support values are Bayesian posterior probabilities.



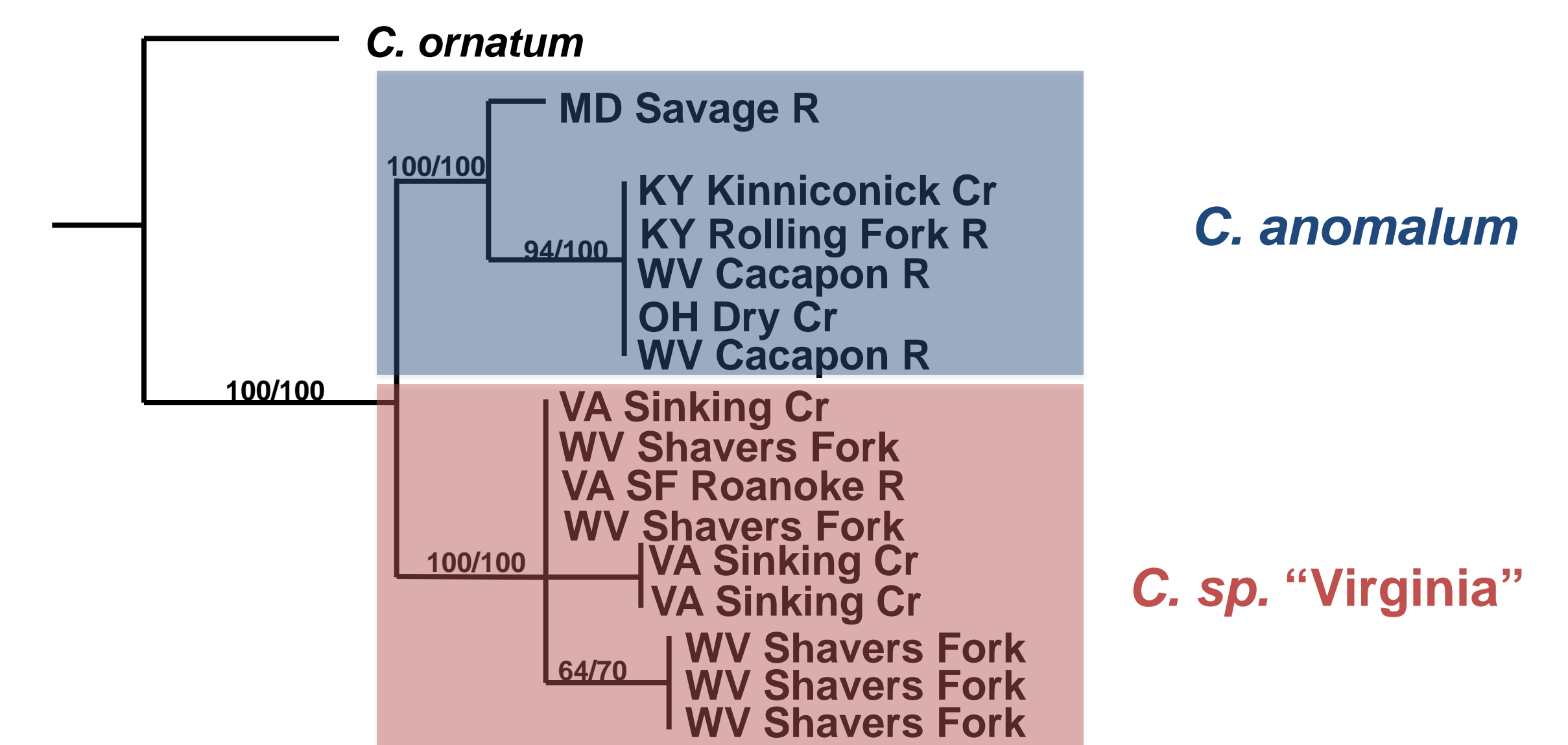
**Figure 2.** Sampling locations for *Campostoma* spp. Shapes distinguish samples from the previous study by Blum et al. (2008) and the current study, and the taxonomic grouping inferred from cytochrome b DNA sequence analysis is indicated by color. The shading indicates the New River drainage, which contains *C. sp.* "Virginia".

## Literature Cited

- Blum MJ, DA Neely, PM Harris, and RL Mayden. 2008. Molecular systematics of the cyprinid genus *Campostoma* (Actinopterygii: Cypriniformes): Disassociation between morphological and mitochondrial differentiation. *Copeia* 2:360-369.
- Goloboff, PA, JS Farris, and KC Nixon. 2008. TNT, a free program for phylogenetic analysis. *Cladistics* 24:774-786.
- Stamatakis, A. 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22:2688-2690.

## Results

- 1,116 bases of cytochrome b sequence data were collected for each of the Cacapon River and Shavers Fork samples.
- Samples from the Cacapon River drainage were assigned to the *C. anomalum* clade (Fig. 3), consistent with the single Potomac River drainage sample (Savage River, MD) analyzed by Blum et al. (2008).
- All Shavers Fork samples were recovered in the *C. sp.* "Virginia" clade characteristic of the New and Roanoke Rivers (Fig. 3).
- Support values for the two clades were very high for both methods of phylogenetic reconstruction.



**Figure 3.** Phylogenetic reconstruction of samples assigned to the *C. anomalum* *C. sp.* "Virginia" clades of Blum et al. (2008) and the Cacapon River and Shavers Fork samples from this study. Support values are MP/ML; clades with values <50 are not indicated.

## Implications/Future Directions

- Results from Cacapon River drainage samples support the conclusion of Blum et al. (2008) that Potomac River basin *Campostoma* are closely related to most Ohio River basin stonerollers (*C. anomalum*).
- Upper Shavers Fork samples group with *C. sp.* "Virginia" of Blum et al. (2008), which was previously thought to be restricted to the New and Roanoke River systems.
- The pattern observed in Shavers Fork is consistent with a hypothesis of historical connections between Shavers Fork and New River drainage waters.
- We plan to expand our sampling in Shavers Fork (testing the hypothesis that the High Falls serve as a barrier between *C. anomalum* and *C. sp.* "Virginia"), and also in adjacent streams to better understand the range of the *C. sp.* "Virginia" haplotype.
- We are in the process of testing for similar phylogeographic patterns between Shavers Fork and the New River drainage in additional taxa.