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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

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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

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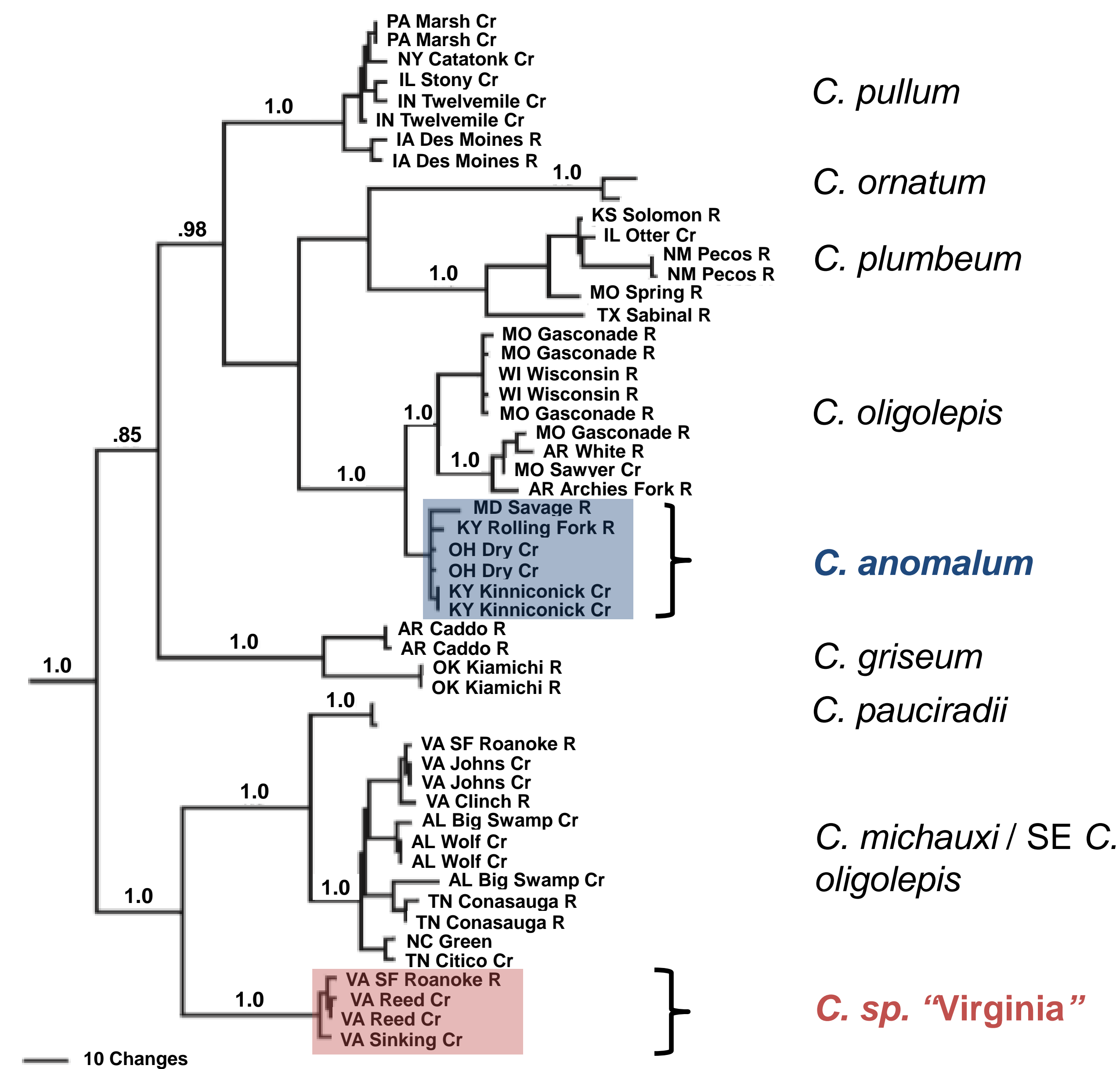


Figure 1. Major lineages within *Campostoma* as identified and presented by Blum et al. (2008). Highlighted lineages are those that occur in West Virginia. Support values are Bayesian posterior probabilities.

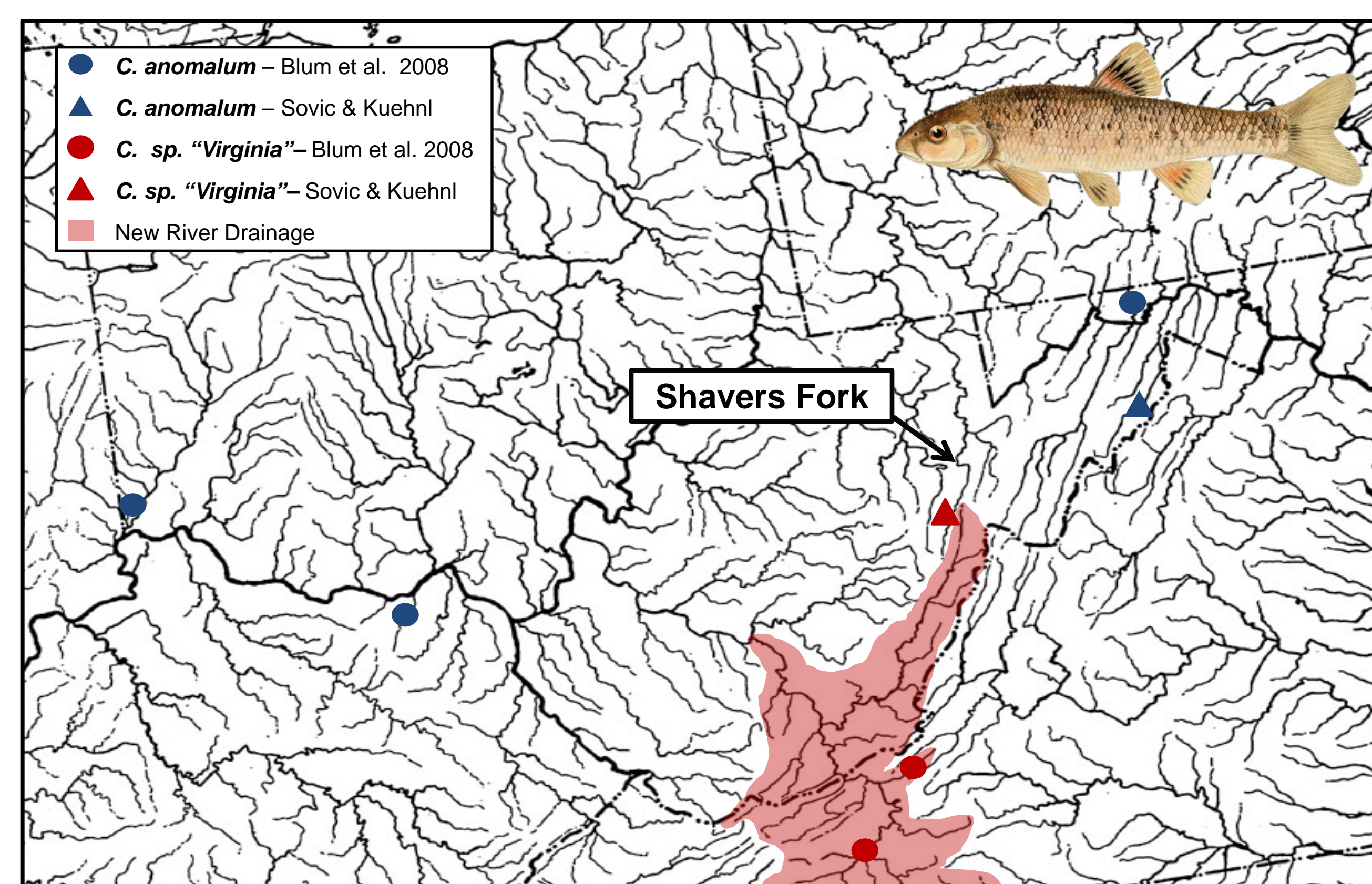


Figure 2. Sampling locations for *Campostoma* spp. Shapes distinguish samples from the previous study by Blum et al. (2008) and the current study, and the taxonomic grouping inferred from cytochrome b DNA sequence analysis is indicated by color. The shading indicates the New River drainage, which contains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

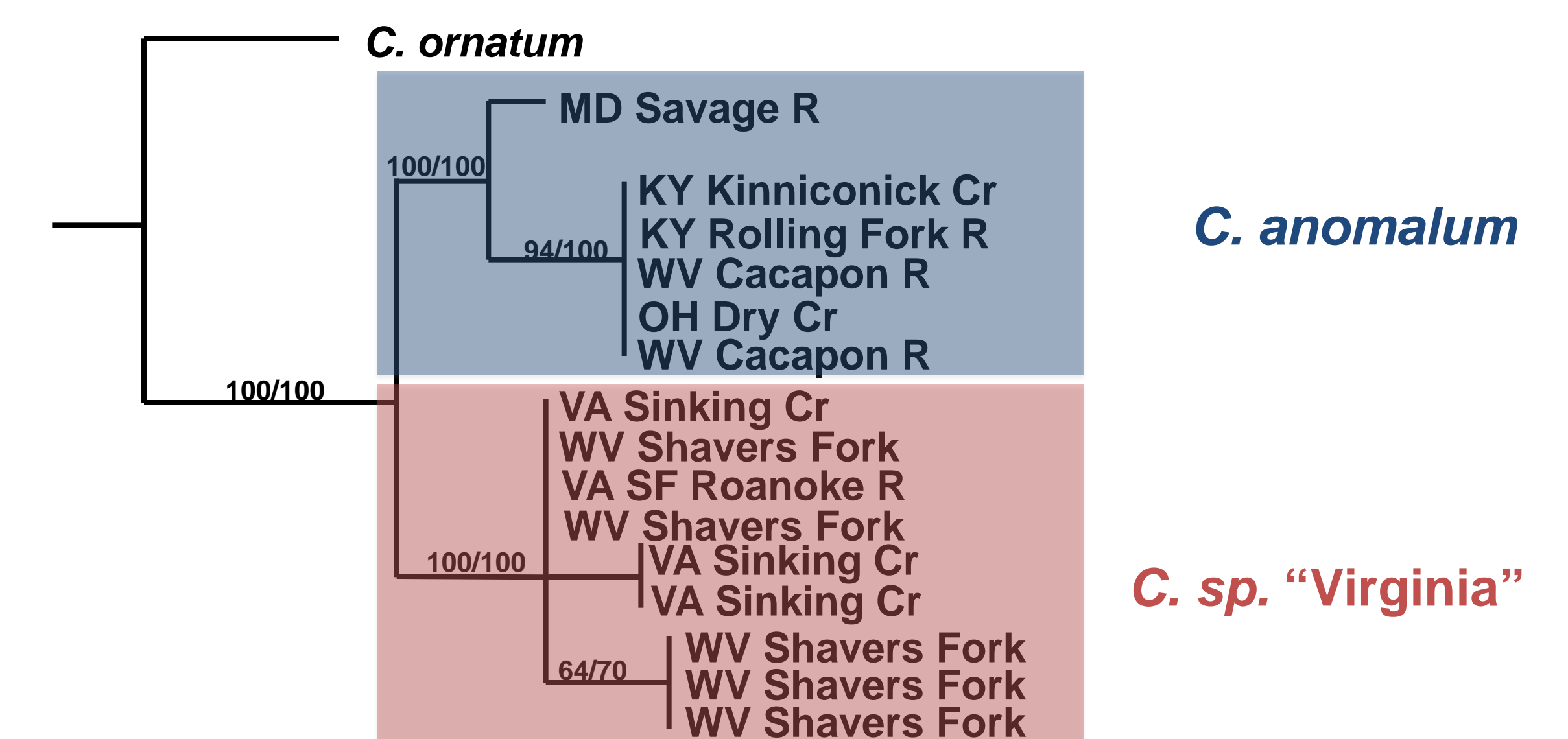


Figure 3. Phylogenetic reconstruction of samples assigned to the *C. anomalum* *C. sp.* "Virginia" clades of Blum et al. (2008) and the Cacapon River and Shavers Fork samples from this study. Support values are MP/ML; clades with values <50 are not indicated.

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.