SUPPORTING INFORMATION

Phylogeography of the Cow Knob Salamander (*Plethodon punctatus*): populations on isolated Appalachian mountaintops are disjunct but not divergent

Frontiers in Amphibian and Reptile Science

Matthew R. Graham, William D. Flint, Alexsis M. Powell, Victor Fet, Thomas K. Pauley

Table S1. Primers to used amplify DNA from *Plethodon punctatus.*

Locus	Primer	Sequence	Source
Cytb	Pglut-F1b	5'-GGTCTGAAAAACCAATGTTGTATTC-3'	Wiens et al. (2006)
	Pthr-R2b	5'-GCCCCCAATTTTTGGYTTACAAG-3'	Wiens et al. (2006)
ND4	ND4(F)	5'-CACCTATGACTACCAAAAGCTCATGTAGAAGC-3'	Arévalo et al. (1994)
	Ephist	5'-TCRTTTTTAGGGTCACRGCCTAG-3'	Wiens et al. (2006)
GAPD	GAPD-F	5'-ACCTTTAATGCGGGTGCTGGCATTGC-3'	Fisher-Reid & Wiens (2011)
	GAPD-R	5'-CATCAAGTCCACAACACGGTTGCTGTA-3'	Fisher-Reid & Wiens (2011)

Literature Cited

Arévalo, E., Davis, S. K., & Sites Jr, J. W. (1994). Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in central Mexico. Systematic Biology, 43(3), 387–418.

Fisher-Reid, M. C., & Wiens, J. J. (2011). What are the consequences of combining nuclear and mitochondrial data for phylogenetic analysis? Lessons from *Plethodon* salamanders and 13 other vertebrate clades. BMC Evolutionary Biology, 11(1), 300.

Wiens, J. J., Engstrom, T. N., & Chippindale, P. T. (2006). Rapid diversification, incomplete isolation, and the "speciation clock" in North American salamanders (genus *Plethodon*): testing the hybrid swarm hypothesis of rapid radiation. Evolution, 60(12), 2585–2603.

Table S2. Prior distributions of parameters used in DIYABC analysis of Cytb from *Plethodon punctatus*.

Interpretation	Parameter	Distribution	Min	Max	Mean	SD	Conditions
Ne Northern (most recent)	N_N	Uniform	10	100,000	–	–	$> N_{NB}$
Ne Southern (most recent)	N_S	Uniform	10	100,000	–	–	$> N_{SB}$
Ne Northern (bottleneck)	N_{NB}	Uniform	10	100,000	–	–	$< N_{NPD}$
Ne Southern (bottleneck)	N_{SB}	Uniform	10	100,000	–	–	$< N_{SPD}$
Ne Northern (post divergence)	N_{NPD}	Uniform	10	100,000	–	–	–
Ne Southern (post divergence)	N_{SPD}	Uniform	10	100,000	–	–	–
Ancestral divergence time*	t_A	Normal	297,100	1,225,000	761,045	236,700	–
Northern expansion time	t_{NE}	Uniform	0	1,225,000	–	–	$< t_{NB}$
Southern expansion time	t_{SE}	Uniform	0	1,225,000	–	–	$< t_{SB}$
Northern bottleneck time	t_{NB}	Uniform	0	1,225,000	–	–	$< t_A$
Southern bottleneck time	t_{SB}	Uniform	0	1,225,000	–	–	$< t_A$
Mutation model (per generation)**	U	Normal	–	–	3.00×10^{-8}	6.71×10^{-9}	HKY

*Divergence time was estimated from the BEAST analysis. Standard deviation (SD) is based on 95% quantiles. Min and Max values are 97.5% quantiles.

**Calculated using mutation rates from Kuchta et al. (2016) (mean 0.00623, SD 0.00149) scaled by a generation time of 4.5 years.

Table S3. Posterior distributions of parameters for Scenario 3 (Series A) from the DIYABC analysis of Cytb samples from throughout the range of *Plethodon punctatus.*

Parameter	Mean	Mode	Median	q05	q95
N_S	202,000	202,000	198,000	113,000	306,000
N_N	227,000	221,000	224,000	129,000	337,000
tA	662,000	668,000	670,000	473,000	823,000
u	1.59×10^{-8}	1.45×10^{-8}	1.57×10^{-8}	1.45×10^{-8}	1.84×10^{-8}

Table S4. Comparison of scenarios tested in the DIYABC analysis. Scenarios with the highest posterior probability in each series (based on a logistic regression estimate), and non-overlapping 95% confidence intervals (in brackets), are highlighted in bold. Note that Scenario 3 had the highest posterior probability of the Series A comparisons, so it was assessed again in Series B as Scenario 1. Thus, the same scenario had the highest posterior probability values in both series.

Scenario	Probability Values
Series A	
Scenario 1	0.0598 [0.0000,0.6582]
Scenario 2	0.1436 [0.0000,0.7887]
Scenario 3	**0.7966 [0.6396,0.9537]**
Series B	
Scenario 1	**0.5747 [0.5480,0.6015]**
Scenario 2	0.1563 [0.1026,0.2099]
Scenario 3	0.2238 [0.1890,0.2586]
Scenario 4	0.0452 [0.0036,0.0867]
Scenario 5	0.0000 [0.0000,0.0000]
Scenario 6	0.0000 [0.0000,0.0000]
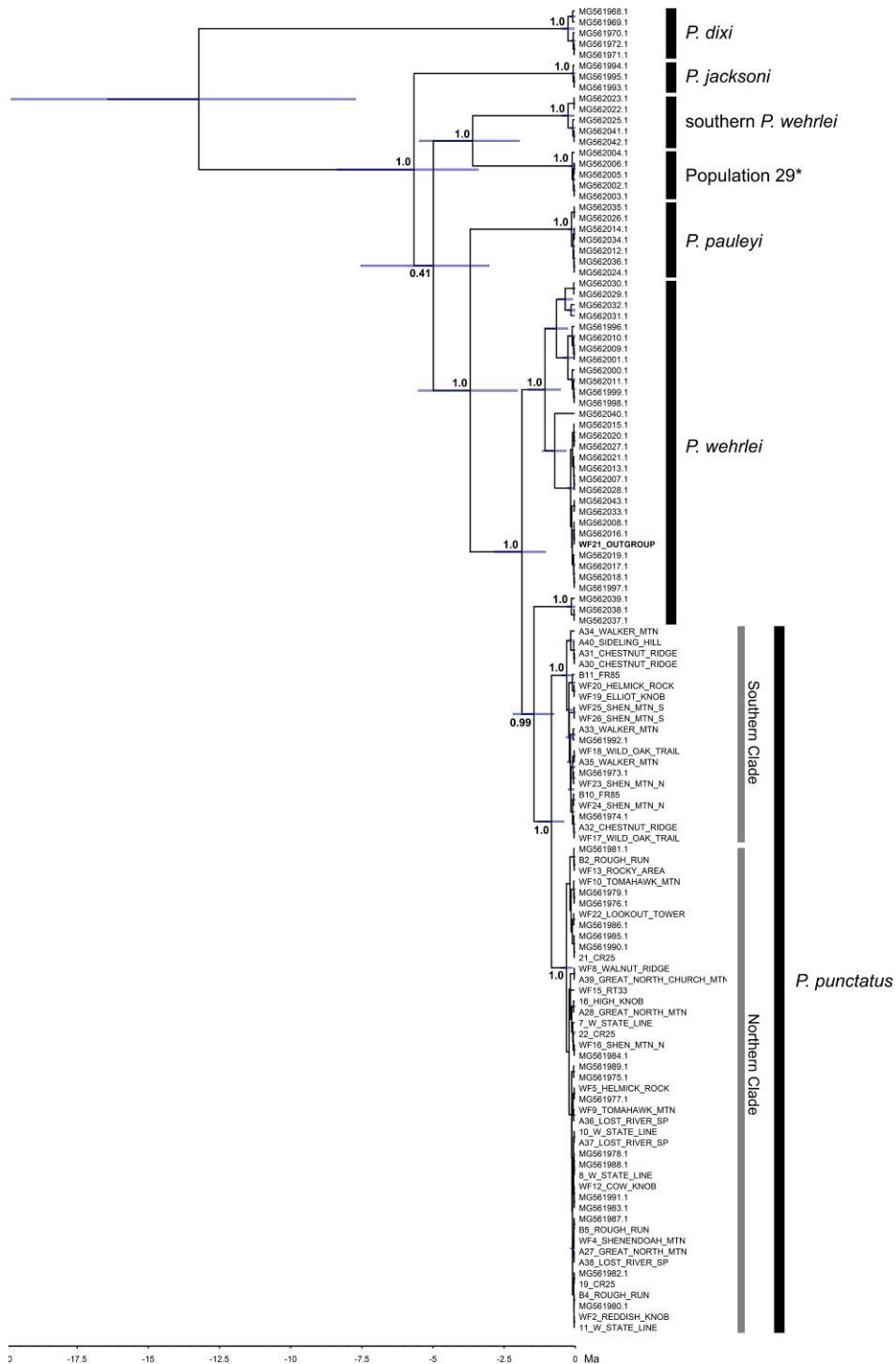
Figure S1. Bayesian chronogram for the *Plethodon wehrlei* species group generated using Cytb data from Kuchta et al. (2018) and this study. The *P. wehrlei* sample in bold was used as an outgroup in our analyses that focused on *P. punctatus.*
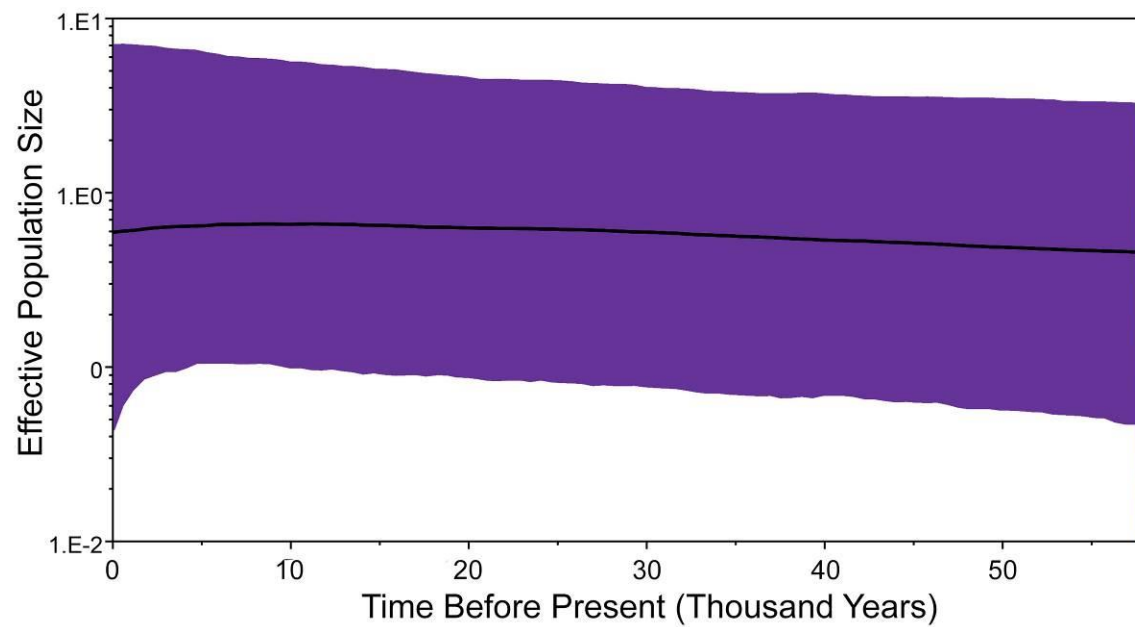
Figure S2. Bayesian skyride plot generated using Cytb sequences from the Southern clade of *Plethodon punctatus*.