





https://doi.org/10.11646/zootaxa.4734.1.1 http://zoobank.org/urn:lsid:zoobank.org:pub:9341AC9B-EB33-4B1B-AC55-836484E65619





Towards rectifying limitations on species delineation in dusky salamanders (*Desmognathus*: Plethodontidae): An ecoregion-drainage sampling grid reveals additional cryptic clades

DAVID A. BEAMER^{1,2} & TRIP LAMB²

¹Department of Natural Science, Nash Community College, Rocky Mount, NC, 27804, USA. ²Department of Biology, East Carolina University, Greenville, NC, USA. E-mail:dbeamer@nashcc.edu



Accepted by M. Vences: 25 Jun. 2019; published: 14 Feb. 2020

DAVID A. BEAMER & TRIP LAMB

Towards rectifying limitations on species delineation in dusky salamanders (*Desmognathus*: Plethodontidae): An ecoregion-drainage sampling grid reveals additional cryptic clades (*Zootaxa* 4734)

61 pp.; 30 cm. 17 Feb. 2020 ISBN 978-1-77670-873-4 (paperback) ISBN 978-1-77670-874-1 (Online edition)

FIRST PUBLISHED IN 2020 BY Magnolia Press P.O. Box 41-383 Auckland 1346 New Zealand e-mail: magnolia@mapress.com https://www.mapress.com/j/zt

© 2020 Magnolia Press

All rights reserved.

No part of this publication may be reproduced, stored, transmitted or disseminated, in any form, or by any means, without prior written permission from the publisher, to whom all requests to reproduce copyright material should be directed in writing.

This authorization does not extend to any other kind of copying, by any means, in any form, and for any purpose other than private research use.

ISSN 1175-5326(Print edition)ISSN 1175-5334(Online edition)

Abstract

Dusky salamanders (Desmognathus) constitute a large, species-rich group within the family Plethodontidae, and though their systematic relationships have been addressed extensively, most studies have centered on particular species complexes and therefore offer only piecemeal phylogenetic perspective on the genus. Recent work has revealed Desmognathus to be far more clade rich—35 reciprocally monophyletic clades versus 22 recognized species—than previously imagined, results that, in turn, provide impetus for additional survey effort within clades and across geographic areas thus far sparsely sampled. We conceived and implemented a sampling regime combining level IV ecoregions and independent river drainages to yield a geographic grid for comprehensive recovery of all genealogically exclusive clades. We sampled over 550 populations throughout the distribution of Desmognathus in the eastern United States of America and generated mitochondrial DNA sequence data (mtDNA; 1,991 bp) for 536 specimens. A Bayesian phylogenetic reconstruction of the resulting haplotypes revealed forty-five reciprocally monophyletic clades, eleven of which have never been included in a comprehensive phylogenetic reconstruction, and an additional three not represented in any molecular systematic survey. Although general limitations associated with mtDNA data preclude new species delineation, we profile each of the 45 clades and assign names to 10 new clades (following a protocol for previous clade nomenclature). We also redefine several species complexes and erect new informal species complexes. Our dataset, which contains topotypic samples for nearly every currently recognized species and most synonymies, will offer a robust framework for future efforts to delimit species within Desmognathus.

Key words: Amphibia, Caudata, *Desmognathus*, mtDNA phylogeny, level IV ecoregion X independent drainage sampling, new clades

Introduction

The species rich salamander family Plethodontidae, with some 475 described species (66% of all salamanders), contributes substantially to vertebrate biodiversity in both tropical and temperate regions of the New World (Rovito *et al.* 2015; Vieites *et al.* 2007). Within the temperate realm, the southeastern United States—long recognized for its plethodontid diversity—accounts for one quarter of all plethodontid species worldwide. Although morphologically distinct species are still described on occasion (Camp *et al.* 2009), much of the Southeast's plethodontid diversity involves cryptic forms ($\approx 40\%$) whose specific status was revealed through population genetic analyses of allozyme data (Anderson & Tilley 2003; Camp *et al.* 2002; Crespi *et al.* 2010; Highton 1979, 1989, 1999; Highton & Peabody 2000; Jacobs 1987; Karlin & Guttman 1981, 1986; Means & Karlin 1989; Tilley 1988; Tilley *et al.* 1978; Tilley & Mahoney 1996; Tilley & Schwerdtfeger 1981). These landmark studies not only identified new species but also revealed complex patterns of gene flow and secondary contact that contribute to the ongoing challenge of plethodontid systematics. Regional cryptic diversity continues to be reported for southeastern plethodontids, now largely on the basis of DNA sequence data (Beamer & Lamb 2008; Kozak *et al.* 2005a; b; Means *et al.* 2017; Steffen *et al.* 2014; Tilley *et al.* 2008, 2013; Timpe *et al.* 2009). These findings justify further inquiry and the need for comprehensive phylogenetic perspectives to understand more fully the intricacies of plethodontid evolution.

Of the Southeast's ten plethodontid genera, perhaps none offers a more intriguing evolutionary history than the dusky salamanders (*Desmognathus*). Traditionally interpreted as a basal group within Plethodontidae, *Desmognathus* is now recognized as a derived clade of relatively recent origin (Chippindale *et al.* 2004; Mueller *et al.* 2004; Titus & Larson 1996)— an unforeseen phylogenetic position that casts the genus in a radically different historical light. *Desmognathus* has become, in fact, the first vertebrate taxon documented to have undergone a major evolutionary reversal in reproductive mode, from direct development to the ancestral biphasic life cycle (free-living aquatic larvae). Moreover, this life history reversal precipitated an adaptive radiation, allowing *Desmognathus* to exploit open lotic niches in communities otherwise established by direct-developing plethodontids (Chippindale *et al.* 2004). Indeed, *Desmognathus* surpasses all other plethodontid genera in its ecological breadth (Bruce 1991; Hairston 1987), which ranges from permanently aquatic riffle specialists to high-altitude terrestrial forms in spruce-fir forests.

Kozak *et al.* (2005b) examined adaptive radiation in *Desmognathus* and provided a compelling case for high rates of clade accumulation subsequent to the taxon's life history reversal. They identified 35 reciprocally monophyletic clades and concluded that current taxonomic interpretations (21-23 spp) woefully underestimate actual species-level diversity. Focusing on the southeastern Coastal Plain, Beamer and Lamb (2008) sampled dusky sala-

mander populations across major independent river drainages and identified several new cryptic clades—countering long-held views that this physiographic region was otherwise species depauperate with respect to *Desmognathus*. These two phylogenetic surveys raise the possibility that additional cryptic species of *Desmognathus* may yet be discovered. If new, unknown clades do exist, what sort of sampling methodology would be most effective in finding them? Here we report a novel geographic sampling regime designed to offer comprehensive recovery of cryptic, reciprocally monophyletic clades within *Desmognathus*. We confirm the presence of additional cryptic clades using this approach and discuss its potential application for other taxonomic groups in the southeastern United States.

Sampling. The success of any phylogenetic survey begins with a sampling regime of appropriate geographic scope and scale. Unfortunately, designing an effective regime can be difficult, due to the vagaries of species distributions. That is, in the absence of prior sampling, no readily apparent approach exists for identifying areas of biogeographical significance beyond broadly recognized geographic boundaries. In the case of morphologically conservative taxa, adequate sampling becomes even more challenging because there is little to suggest where the most informative collections should be made. Adequate sampling can be exacerbated even further by morphologically conservative taxa that occupy large geographic ranges. Dusky salamanders are exemplary in this respect: they are a morphologically conservative taxon distributed throughout the eastern United States of America (Fig. 1). Not only do dusky salamanders span a large geographic area, they also occupy a wide variety of habitats (Beamer & Lamb 2008; Bruce 1991; Chippindale *et al.* 2004; Hairston 1949, 1987; Kozak *et al.* 2005b; Organ 1961; Titus & Larson 1996). Although most species are semi-aquatic inhabitants of small streams, others (as noted previously) range from fully aquatic to completely terrestrial. Moreover, certain species demonstrate habitat shifts along elevation gradients or in the presence of congeners (Rissler *et al.* 2004). Simply put, *Desmognathus* has an enormous distribution and occupies a multitude of habitats.



FIGURE 1. Collection localities.

Although dusky salamanders can be unequivocally assigned to genus, species assignments have proven far more difficult; their brown-colored but otherwise nondescript habitus lead to a prominent field guide's claim that "identifying these salamanders is like working with fall warblers, only worse" (Conant & Collins 1998). Such

pronounced morphological conservatism has lead many workers to choose molecular genetic approaches for species delimitation as well as other aspects of systematics. Most surveys have focused on morphological species complexes, which is problematic because homoplasy is well documented for salamanders (Wake 1991), and recent phylogenetic surveys have questioned the validity of the traditionally-recognized species complexes in *Desmognathus* (Beamer & Lamb 2008; Kozak *et al.* 2005b; Tilley *et al.* 2008, 2013).

Given homoplasy's prevalence in salamander evolution, especially in morphologically conservative taxa (Wake 2009), species-level phylogenetic surveys should entail a broadly-based yet fine-scaled sampling effort. This approach is essential for plethodontid clades prone to ecotypic evolution and cryptic species diversity (Anderson & Tilley 2003; Bingham *et al.* 2018; Camp *et al.* 2002; García-París *et al.* 2000, 2008; Highton 1989, 1999; Highton & Peabody 2000; Jockusch *et al.* 2001, 2012; Jockusch & Wake 2002; Parra-Olea & Wake 2001; Tilley *et al.* 1978, 2008, 2013; Tilley & Mahoney 1996).



FIGURE 2. Color-coded profile of the Level IV Ecoregions present in the Pee Dee and Santee River drainages; the number of dusky salamander species present in each level IV ecoregion X drainage sample unit (based on current distribution maps) is shown.

What geologic and/or physiographic features are particularly relevant to the ecology and evolution of *Desmognathus*? Upland dispersal is probably minimal; instead, most inter-population movement likely occurs via streamside/wetland conduits, which are eventually circumscribed by a given river drainage system (i.e., watersheds entering the ocean independently of others). Many dusky species are distributed across multiple river drainages, so sampling across watersheds is clearly important. Dry divides between adjacent drainages and the obvious barrier posed by estuarine coastal wetlands have likely played important roles in structuring contemporary and historical patterns of gene flow. Indeed, the influence of river drainages on phylogeography and speciation in semi-aquatic species is well established and includes several examples for dusky salamanders (Beamer & Lamb 2008; Jones *et al.* 2006; Kozak *et al.* 2005a; b; Voss *et al.* 1995). We consider independent river drainages to be the single most relevant geographic focal point and, thus, a key sampling unit for investigating evolutionary relationships in *Desmognathus*. Certain independent river drainages with headwaters near the coast (e.g., Tar River in NC) tend to be occupied by just one or two dusky species. However, many of the sampled drainages cover large portions of the landscape and cross multiple habitats from their headwaters to the sea. The various habitats within these larger systems are often occupied by several species of *Desmognathus*, with some drainages containing up to thirteen species (Bruce 1991). This pattern suggests other geographic components may contribute relevant ecological variation that, in turn, may influence evolutionary processes. For additional resolution, we have identified a second sampling focal point to address fine scale geographic coverage, level IV ecoregions. Level IV ecoregions are geographic units delineated by the US Environmental Protection Agency that denote general ecosystem similarities as well as commonality in the type, quality and quantity of resources available. These ecoregions often correspond to physiographic provinces delineated by previous research groups (Fenneman 1938).

We argue that sampling every level IV ecoregion within each independent river drainage across the entire range extent of *Desmognathus* should provide a transect grid of appropriate scale to detect any and all cryptic evolutionary clades. In the event that a previously unknown clade is missed in a particular ecoregion/drainage sample, it will likely be sampled in an adjacent ecoregion upstream or downstream. Moreover, the range for any given clade should be sampled adequately to effectively delimit the range extent of that clade.

The large number of independent river drainages and level IV ecoregions across the study area precludes illustrating all of the sample units. However we have illustrated the scale of level IV ecoregions sampling in a pair of adjacent drainages that head in the Appalachian Mountains and drain into the Atlantic Ocean (Fig. 2).

Methods

Ecoregion X drainage sampling. We attempted to collect specimens for every level IV ecoregion within each independent river drainage—providing sampling units hereafter termed ecoregion X drainage site—across the entire distribution of *Desmognathus*. Extreme northern portions of the range extent were sampled more sparsely given lower species numbers and limited genetic diversity characterizing this recently glaciated region (Karlin & Guttman 1986; Tilley & Mahoney 1996). We attempted to collect 5–6 individuals per species known to occur within each unit, yielding a total of 5045 specimens representing 312 ecoregion X drainage sites (Fig. 1).

We generated DNA sequence data for 536 specimens representing 179 ecoregion X drainage sites. The discrepancy (n = 357) between specimen and site numbers is due to the fact that many sites were represented by multiple species. In only a few cases were sequence data generated for multiple individuals of one species at a single site. Importantly, each of the 22–24 currently recognized species of *Desmognathus* (Frost 2014; Tilley *et al.* 2012) are represented by topotypic or near topotypic specimens (see comment in *ocoee* complex). In addition we secured topotypic samples for the following synonymized taxa: *D. chermocki*, *D. marmoratus intermedia*, *D. marmoratus roboratus*, *D. monticola jeffersoni*, *D. perlapsus*, and *D. quadramaculatus amphileucus*, which represent all of the synonyms for *Desmognathus* except those assigned to populations of *D. fuscus*. Most of the synonymized names for *D. fuscus* represent populations from the vicinities of New York City, Philadelphia, and New Jersey, and likely do not represent cryptic diversity. We searched but were unable to collect topotypic material for *D. phoca* (a junior synonym for *fuscus* from Ohio), which may represent *D. monticola* (Graziano & Reid 2006), although their record has been since rejected (Matson *et al.* 2010).

Amplification and sequencing. Genomic DNA was extracted from tail tips (stored in 95% ethanol or RNAlater) using Qiagen's DNeasy kits. We sequenced a 1,991 bp segment of the mitochondrial genome spanning the entire NADH dehydrogenase subunit 2 (*ND2*) gene, five tRNA genes (tRNA^{Trp}, tRNA^{Ala}, tRNA^{Asn}, tRNA^{Cys} and tRNA^{Tyr}), and the first half of the cytochrome oxidase subunit c (*COX1*) gene, given this region's demonstrated phylogenetic performance for both intraspecific and species-level comparisons in amphibians (Mueller *et al.* 2004) and use in previous dusky salamander surveys (Beamer & Lamb 2008; Jones *et al.* 2006; Kozak *et al.* 2005b; Rissler *et al.* 2004; Rissler & Taylor 2003). This fragment was amplified with previously published primers (Beamer & Lamb 2008; Kozak *et al.* 2005b) used in a different combination to produce a larger amplicon. Amplifications were completed in a total volume of 25 µl, with a denaturation at 94°C (60 s), annealing at 52°C (45 s), and extension at 68°C (90 s) for a total of 35 cycles. PCR products were purified using ExoSap-IT (USB Corp) and sequenced in both directions on an ABI 3130 Genetic Analyzer using dye-labeled terminators (BigDyeTM).

Specimen #	Genbank Accession #	Species	mtDNA clade	Locality	Latitude	Longitude	State	County
DAB4237	MH403766	conanti	conanti E	1	31.24472	-92.67917	LA	Rapides
DAB2589	MH403820	conanti	conanti E	2	31.74298	-93.11606	LA	Natchitoches
DAB647	MH403768	conanti	conanti E	3	31.56740	-92.51120	LA	Grant
DAB4209	MH403767	conanti	conanti E	4	32.05133	-92.90968	LA	Natchitoches
DAB2225	MH403816	conanti	conanti B	5	30.82200	-91.42320	LA	West Feliciana
DAB4677	MH403817	conanti	conanti B	6	31.06838	-91.51675	MS	Wilkinson
DAB2436	MH403799	conanti	conanti E	7	31.83965	-91.75695	LA	Catahoula
DAB2239	MH403819	conanti	conanti B	8	30.71220	-90.48010	LA	Tangipahoa
DAB2198	MH403818	conanti	conanti B	9	31.12693	-90.84651	MS	Amite
DAB2650	MH403815	conanti	conanti B	10	31.42600	-90.98507	MS	Franklin
DAB4210	MH403593	brimleyorum	brimleyorum	11	34.37745	-93.87959	AR	Montgomery
DAB2452	MH403595	valentinei	valentinei	12	30.57875	-89.92999	LA	St. Tammany
DAB4221	MH403591	brimleyorum	brimleyorum	13	34.66179	-93.97694	AR	Polk
DAB2205	MH403828	conanti	conanti C	14	30.94200	-89.97830	LA	Washington
DAB4227	MH403594	brimleyorum	brimleyorum	15	33.75180	-92.69134	AR	Ouachita
DAB2136	MH404079	brimleyorum	brimleyorum	16	34.54400	-93.02330	AR	Garland
DAB2228	MH403592	brimleyorum	brimleyorum	16	34.54400	-93.02330	AR	Garland
DAB2645	MH404091	conanti	conanti C	17	31.59166	-90.06607	MS	Lawrence
DAB4720	MH403597	valentinei	valentinei	18	30.66697	-89.13144	MS	Harrison
DAB2634	MH403846	conanti	conanti D	19	32.54746	-90.71488	MS	Warren
DAB4686	MH403829	conanti	conanti C	20	31.16034	-89.24463	MS	Forrest
DAB4338	MH403618	valentinei	valentinei	21	30.45518	-88.32713	AL	Mobile
DAB438	MH403825	conanti	conanti C	22	31.95755	-89.26715	MS	Jasper
DAB4350	MH403596	valentinei	valentinei	23	31.61589	-88.88201	MS	Wayne
DAB2196	MH403824	conanti	conanti C	24	31.89590	-89.14490	MS	Jasper
DAB346	MH403832	conanti	conanti C	25	31.03120	-87.84063	AL	Baldwin
DAB2336	MH403831	conanti	conanti C	26	31.09452	-87.83271	AL	Baldwin
DAB2641	MH403823	conanti	conanti C	27	32.27677	-88.85118	MS	Lauderdale
DAB2337	MH403933	monticola	monticola A	28	31.40190	-87.87582	AL	Clarke
DAB2594	MH403822	conanti	conanti C	29	30.50903	-86.91340	FL	Santa Rosa
DAB347	MH403834	conanti	conanti C	30	30.95803	-87.21200	FL	Santa Rosa

TABLE 1. Sample localities and GenBank accession #'s.

TABLE 1. (Continued)										
Specimen #	Genbank Accession #	Species	mtDNA clade	Locality	Latitude	Longitude	State	County		
DAB2316	MH403826	conanti	conanti C	31	31.09556	-86.54031	AL	Covington		
DAB2321	MH403827	conanti	conanti C	32	31.09785	-86.53544	AL	Covington		
DAB435	MH403821	conanti	conanti C	33	30.46497	-85.86397	FL	Washington		
DAB345	MH403833	conanti	conanti C	34	31.57220	-86.73821	AL	Butler		
DAB2323	MH403830	conanti	conanti C	35	31.19298	-85.85845	AL	Geneva		
DAB218	MH403923	apalachicolae	apalachicolae	36	30.51085	-84.95792	FL	Liberty		
DAB4674	MH403578	aeneus	aeneus	37	32.96612	-87.31616	AL	Bibb		
DAB929	MH403843	conanti	conanti D	38	33.21749	-87.44254	AL	Tuscaloosa		
DAB911	MH403579	aeneus	aeneus	39	33.25730	-87.37304	AL	Tuscaloosa		
DAB860	MH403924	apalachicolae	apalachicolae	40	30.44890	-84.47510	FL	Leon		
DAB2443	MH403927	apalachicolae	apalachicolae	41	31.30557	-85.07901	GA	Early		
DAB1972	MH403838	conanti	conanti D	42	34.09305	-87.61473	AL	Winston		
DAB3782	MH403847	conanti	conanti D	43	35.39711	-88.84305	TN	Hardeman		
DAB4369	MH403848	conanti	conanti D	43	35.39711	-88.84305	TN	Hardeman		
DAB4258	MH403858	conanti	conanti D	44	34.67394	-87.98419	AL	Colbert		
DAB924	MH403839	conanti	conanti D	45	34.34220	-87.39322	AL	Lawrence		
DAB1403	MH403926	apalachicolae	apalachicolae	46	32.03959	-84.88990	GA	Stewart		
DAB1418	MH403841	conanti	conanti D	47	33.33684	-86.02213	GA	Bibb		
DAB1420	MH403842	monticola	monticola C	47	33.33684	-86.02213	GA	Bibb		
DAB3701	MH403849	conanti	conanti D	48	35.82806	-88.32579	TN	Carroll		
DAB4680	MH403929	monticola	monticola C	49	33.48497	-85.93107	AL	Talladega		
DAB2805	MH403928	monticola	monticola C	50	33.48362	-85.91096	AL	Talladega		
DAB4672	MH403569	aeneus	aeneus	51	33.47169	-85.82326	AL	Cleburne		
DAB1397	MH403925	apalachicolae	apalachicolae	52	32.30705	-84.58447	GA	Marion		
DAB4257	MH403845	conanti	conanti D	53	34.06980	-86.31245	AL	Etowah		
DAB349	MH403587	auriculatus	auriculatus A	54	30.19202	-82.42716	FL	Baker		
DAB2814	MH403930	monticola	monticola C	55	33.52663	-85.72684	AL	Cleburne		
DAB3830	MH403850	conanti	conanti D	56	35.85308	-87.96389	TN	Benton		
DAB2224	MH403844	conanti	conanti D	57	35.78740	-87.87000	TN	Perry		
DAB2338	MH403605	ocoee	ocoee D	58	32.83597	-84.89367	GA	Harris		

8 · Zootaxa 4734 (1) © 2020 Magnolia Press

BEAMER

TABLE 1. (Continued)									
Specimen #	Genbank Accession #	Species	mtDNA clade	Locality	Latitude	Longitude	State	County	
DAB1406	MH403920	apalachicolae	apalachicolae	59	32.17245	-84.20913	GA	Schley	
DAB1411	MH403921	apalachicolae	apalachicolae	59	32.17245	-84.20913	GA	Schley	
DAB1385	MH403586	auriculatus	auriculatus A	60	31.03617	-82.88000	GA	Clinch	
DAB1380	MH403604	apalachicolae	apalachicolae	61	32.5065	-84.33030	GA	Taylor	
DAB4186	MH403861	conanti	conanti D	62	34.09962	-85.90407	AL	Etowah	
DAB4673	MH403570	aeneus	aeneus	63	32.88106	-84.50327	GA	Talbot	
DAB2339	MH403610	ocoee	ocoee D	64	32.80616	-84.40547	GA	Talbot	
DAB1394	MH403919	apalachicolae	apalachicolae	65	32.43632	-84.02044	GA	Crawford	
DAB2308	MH403857	conanti	conanti D	66	34.77558	-86.32147	AL	Jackson	
DAB2334	MH403856	conanti	conanti D	67	34.76881	-86.31333	AL	Jackson	
DAB2344	MH403855	conanti	conanti D	68	33.95465	-85.2903	GA	Polk	
DAB3808	MH403852	conanti	conanti D	69	37.10489	-88.40123	KY	Livingston	
DAB222	MH403851	conanti	conanti D	70	37.10670	-88.40256	KY	Livingston	
DAB3771	MH403737	fuscus	fuscus A	71	36.70930	-87.83290	TN	Trigg	
DAB2322	MH403606	ocoee	ocoee D	72	33.47078	-84.38362	GA	Clayton	
DAB3856	MH403854	conanti	conanti D	73	36.16257	-87.05183	TN	Cheatham	
DAB2311	MH403609	ocoee	ocoee D	74	33.73937	-84.62799	GA	Douglas	
DAB3889	MH404022	ocoee	ocoee FGH	75	34.83896	-85.64010	AL	Jackson	
DAB3702	MH403840	conanti	conanti D	76	35.45342	-86.22459	TN	Coffee	
DAB3735	MH403853	conanti	conanti D	77	35.45167	-86.22145	TN	Coffee	
DAB3792	MH403836	conanti	conanti D	78	35.45001	-86.21974	TN	Coffee	
DAB4351	MH403837	conanti	conanti D	78	35.45001	-86.21974	TN	Coffee	
DAB4682	MH403945	monticola	monticola B	79	34.51436	-85.07310	GA	Gordon	
DAB1412	MH403931	monticola	monticola B	80	34.56487	-85.10309	GA	Floyd	
DAB1414	MH403859	conanti	conanti D	80	34.56487	-85.10309	GA	Floyd	
DAB3871	MH403742	fuscus	fuscus A	81	35.24986	-85.75073	TN	Grundy	
DAB4353	MH403617	abditus	abditus	82	35.24974	-85.75051	TN	Grundy	
DAB4767	MH404087	conanti	conanti D	83	34.25239	-84.68802	AL	Bartow	
DAB2580	MH403589	auriculatus	auriculatus A	84	31.49951	-81.91082	GA	Wayne	
DAB3763	MH403736	conanti	conanti D	85	35.76554	-86.08018	TN	Cannon	

TABLE 1. (Con	TABLE 1. (Continued)									
Specimen #	Genbank Accession #	Species	mtDNA clade	Locality	Latitude	Longitude	State	County		
DAB4766	MH403781	conanti	conanti A	86	32.65536	-82.96806	GA	Laurens		
DAB3845	MH404021	ocoee	ocoee FGH	87	35.16272	-85.40957	TN	Marion		
DAB867	MH403783	conanti	conanti A	88	31.66659	-81.84647	GA	Wayne		
DAB2324	MH403860	conanti	conanti D	89	34.94510	-84.78028	GA	Murray		
DAB4787	MH403941	monticola	monticola B	90	34.86164	-84.64236	GA	Murray		
DAB4795	MH403573	aeneus	aeneus	90	34.86164	-84.64236	GA	Murray		
DAB4809	MH403574	aeneus	aeneus	91	34.81261	-84.55876	GA	Gilmer		
DAB4810	MH404056	folkertsi	folkertsi	92	34.81490	-84.56065	GA	Gilmer		
DAB4817	MH403942	monticola	monticola B	92	34.81490	-84.56065	GA	Gilmer		
DAB4835	MH404088	conanti	conanti D	93	34.94083	-84.66549	GA	Murray		
DAB4839	MH403577	aeneus	aeneus	93	34.94083	-84.66549	GA	Murray		
DAB4893	MH403575	aeneus	aeneus	94	34.89149	-84.51340	GA	Fannin		
DAB4896	MH403576	aeneus	aeneus	94	34.89149	-84.51340	GA	Fannin		
DAB2153	MH403741	fuscus	fuscus A	95	36.14340	-85.75350	TN	Putnam		
DAB4404	MH403775	conanti	conanti A	96	33.70072	-83.28643	GA	Greene		
DAB348	MH403590	auriculatus	auriculatus A	97	32.07861	-81.66000	GA	Bryan		
DAB1391	MH403588	auriculatus	auriculatus A	97	32.07861	-81.66000	GA	Bryan		
DAB4592	MH403786	conanti	conanti A	98	33.03853	-82.60830	GA	Washington		
DAB4663	MH403787	conanti	conanti A	99	33.22926	-82.68813	GA	Glascock		
DAB1806	MH403943	monticola	monticola B	100	35.11061	-84.56472	TN	Polk		
DAB4273	MH403936	monticola	monticola B	101	35.07010	-84.46941	TN	Polk		
DAB4352	MH403932	monticola	monticola B	101	35.07010	-84.46941	TN	Polk		
DAB4830	MH403584	aeneus	aeneus	102	34.81289	-84.18617	GA	Fannin		
DAB4832	MH403944	monticola	monticola B	102	34.81289	-84.18617	GA	Fannin		
DAB1287	MH404063	quadramaculatus	quadramaculatus A	103	34.6434	-83.97670	GA	Lumpkin		
DAB1346	MH403935	monticola	monticola B	104	34.62690	-83.95940	GA	Lumpkin		
DAB1348	MH403940	monticola	monticola B	104	34.62690	-83.95940	GA	Lumpkin		
DAB1350	MH403571	aeneus	aeneus	104	34.62690	-83.95940	GA	Lumpkin		
DAB1352	MH403922	apalachicolae	apalachicolae	104	34.62690	-83.95940	GA	Lumpkin		
DAB4671	MH403572	aeneus	aeneus	105	35.2282	-84.54859	TN	Polk		

TABLE 1. (Continued)										
Specimen #	Genbank Accession #	Species	mtDNA clade	Locality	Latitude	Longitude	State	County		
DAB4769	MH404058	marmoratus	marmoratus B	106	34.64158	-83.94225	GA	Lumpkin		
DAB1807	MH404065	quadramaculatus	quadramaculatus A	107	34.63407	-83.93454	GA	Lumpkin		
DAB4842	MH403934	monticola	monticola B	108	34.75272	-84.01368	GA	Union		
DAB4850	MH403580	aeneus	aeneus	109	34.75349	-84.01283	GA	Union		
DAB4857	MH403917	apalachicolae	apalachicolae	109	34.75349	-84.01283	GA	Union		
DAB1421	MH403640	auriculatus	auriculatus B	110	32.05297	-81.28073	GA	Chatham		
DAB3767	MH403745	fuscus	fuscus A	111	35.67957	-84.89058	TN	Rhea		
DAB950	MH403938	monticola	monticola B	112	34.76780	-83.94650	GA	Union		
DAB951	MH403918	apalachicolae	apalachicolae	112	34.76780	-83.94650	GA	Union		
DAB1178	MH404064	quadramaculatus	quadramaculatus A	112	34.76780	-83.94650	GA	Union		
DAB1251	MH403939	monticola	monticola B	112	34.76780	-83.94650	GA	Union		
DAB1256	MH403937	apalachicolae	apalachicolae	112	34.76780	-83.94650	GA	Union		
DAB3721	MH403743	fuscus	fuscus A	113	35.73099	-84.86357	TN	Rhea		
DAB3730	MH403615	abditus	abditus	113	35.73099	-84.86357	TN	Rhea		
DAB3714	MH403740	fuscus	fuscus A	114	35.69571	-84.80450	TN	Rhea		
DAB4396	MH403774	conanti	conanti A	115	33.88606	-82.99187	GA	Oglethorpe		
DAB4678	MH404066	quadramaculatus	quadramaculatus A	116	34.713078	-83.79103	GA	White		
DAB4679	MH404054	folkertsi	folkertsi	116	34.713078	-83.79103	GA	White		
DAB1386	MH403784	conanti	conanti A	117	33.23883	-82.29807	GA	Jefferson		
DAB1434	MH403785	conanti	conanti A	117	33.23883	-82.29807	GA	Jefferson		
DAB4695	MH404073	quadramaculatus	quadramaculatus A	118	34.55510	-83.54021	GA	Habersham		
DAB4406	MH403773	conanti	conanti A	119	34.20467	-83.14784	GA	Madison		
DAB2144	MH403744	fuscus	fuscus A	120	35.88440	-84.82610	TN	Cumberland		
DAB1387	MH403795	conanti	conanti A	121	32.34842	-81.24235	GA	Effingham		
DAB2548	MH403796	conanti	conanti A	121	32.34842	-81.24235	GA	Effingham		
DAB2012	MH403647	auriculatus	auriculatus B	122	32.20820	-81.09520	SC	Jasper		
DAB2062	MH403649	auriculatus	auriculatus B	122	32.20820	-81.09520	SC	Jasper		
DAB4865	MH403581	aeneus	aeneus	123	34.84870	-83.59714	GA	Rabun		
DAB1821	MH403616	abditus	abditus	124	36.05940	-84.79430	TN	Cumberland		
DAB1802	MH403797	conanti	conanti A	125	32.49540	-81.21270	SC	Jasper		

TABLE 1. (Continued)

33798 c 33790 c 33791 c 33792 c 33792 c 33805 s 33794 c 33946 n	conanti conanti conanti conanti monticola santeetlah conanti	conanti A conanti A conanti A conanti A monticola B santeetlah	125 126 126 126 127	32.49540 33.24134 33.24134 33.24134 35.14732	-81.21270 -81.94831 -81.94831 -81.94831 -83.83147	SC GA GA GA NC	Jasper Burke Burke Glue L
13790 c 13791 c 13792 c 14002 m 13805 s 13794 c 13946 m	conanti conanti conanti monticola santeetlah conanti	conanti A conanti A conanti A monticola B santeetlah	126 126 126 127	33.24134 33.24134 33.24134 35.14732	-81.94831 -81.94831 -81.94831 -83.83147	GA GA GA NC	Burke Burke Gland
13791 c 13792 c 13792 r 14002 r 13805 s 13794 c 13946 r	conanti conanti monticola santeetlah conanti	conanti A conanti A monticola B santeetlah	126 126 127	33.24134 33.24134 35.14732	-81.94831 -81.94831 -83.83147	GA GA NC	Burke
03792 c 04002 m 03805 s 03794 c 03946 m	conanti monticola santeetlah conanti	conanti A monticola B santeetlah	126 127 128	33.24134 35.14732	-81.94831 -83.83147	GA NC	Burke
04002 m 03805 s 03794 c 03946 m	monticola santeetlah conanti	monticola B santeetlah	127	35.14732	-83.83147	NC	C1 1
)3805 s)3794 c)3946 n	santeetlah conanti	santeetlah	128				Cherokee
)3794 c)3946 m	conanti	1° A	120	35.34784	-84.02856	NC	Graham
)3946 n	. 1	conanti A	129	33.08654	-81.76446	GA	Burke
	monticola	monticola B	130	35.40620	-84.08142	TN	Monroe
)3947 n	monticola	monticola B	130	35.40620	-84.08142	TN	Monroe
03608 0	ocoee	ocoee D	131	34.72990	-83.38580	GA	Habersham
04055 f	folkertsi	folkertsi	132	34.66750	-83.31630	GA	Stephens
04007 g	quadramaculatus	quadramaculatus A	132	34.66750	-83.31630	GA	Stephens
)3607 d	ocoee	ocoee D	132	34.66750	-83.31630	GA	Stephens
04008 n	monticola	monticola B	133	34.67172	-83.31433	SC	Oconee
03603 a	ocoee	ocoee D	133	34.67172	-83.31433	SC	Oconee
04078 g	quadramaculatus	quadramaculatus A	134	34.69454	-83.32927	SC	Oconee
04024 a	ocoee	ocoee FGH	135	35.34768	-83.97356	NC	Graham
)4067 g	quadramaculatus	quadramaculatus A	135	35.34768	-83.97356	NC	Graham
)3585 a	aeneus	aeneus	135	35.34768	-83.97356	NC	Graham
)3652 a	auriculatus	auriculatus B	136	32.58800	-81.20197	SC	Jasper
)3738 f	fuscus	fuscus A	137	35.92794	-84.54158	TN	Roane
)3994 n	monticola	monticola B	138	35.35689	-83.93420	NC	Graham
)3835 c	conanti	conanti D	139	35.46162	-84.03355	TN	Monroe
04015 d	ocoee	ocoee E	140	34.99358	-83.55858	NC	Clay
)3793 c	conanti	conanti A	141	33.06966	-81.62738	GA	Burke
)3788 c	conanti	conanti A	141	33.06966	-81.62738	GA	Burke
)4045 n	marmoratus	quadramaculatus/mar-	142	35.25579	-83.80502	NC	Graham
		moratus C					
04068 g	quadramaculatus	quadramaculatus A	142	35.25579	-83.80502	NC	Graham
	3946 3947 3608 4055 4007 3607 4008 3603 4078 4024 4067 3585 3652 3738 3994 3835 4015 3793 3788 4045	3946monticola3947monticola3608ocoee4055folkertsi4007quadramaculatus3607ocoee4008monticola3603ocoee4078quadramaculatus4024ocoee4067quadramaculatus3585aeneus3652auriculatus3738fuscus3994monticola3835conanti4015ocoee3793conanti4045marmoratus	3946monticolamonticola B3947monticolamonticola B3608ocoeeocoee D4055folkertsifolkertsi4007quadramaculatusquadramaculatus A3607ocoeeocoee D4008monticolamonticola B3603ocoeeocoee D4078quadramaculatusquadramaculatus A4024ocoeeocoee FGH4067quadramaculatusquadramaculatus A3585aeneusaeneus3652auriculatusauriculatus B3738fuscusfuscus A3994monticolamonticola B3738conanticonanti D4015ocoeeocoee E3793conanticonanti A3788conanticonanti A4045marmoratusquadramaculatus A4068quadramaculatusquadramaculatus A	3946monticolamonticola B1303947monticolamonticola B1303947monticolamonticola B1303608ocoeeocoee D1314055folkertsifolkertsi1324007quadramaculatusquadramaculatus A1323607ocoeeocoee D1324008monticolamonticola B1333603ocoeeocoee D1334078quadramaculatusquadramaculatus A1344024ocoeeocoee FGH1354067quadramaculatusquadramaculatus A1353585aeneusaeneus1353652auriculatusquadramaculatus B1363738fuscusfuscus A1373994monticolamonticola B1383835conanticonanti D1394015ocoeeocoee E1403793conanticonanti A1414045marmoratusquadramaculatus/mar-1424068quadramaculatusquadramaculatus A142	3946 monticola monticola B 130 35.40620 3947 monticola monticola B 130 35.40620 3608 ocoee ocoee D 131 34.72990 4055 folkertsi folkertsi 132 34.66750 4007 quadramaculatus quadramaculatus A 132 34.66750 3608 monticola monticola B 133 34.67172 4008 monticola monticola B 133 34.67172 3603 ocoee ocoee D 133 34.67172 4078 quadramaculatus quadramaculatus A 134 34.69454 4024 ocoee ocoee FGH 135 35.34768 3585 aeneus aeneus 135 35.34768 3652 auriculatus quadramaculatus A 136 32.58800 3738 fuscus fuscus A 137 35.92794 3994 monticola monticola B 138 35.35689 3753	3946 monticola monticola B 130 35.40620 -84.08142 3947 monticola monticola B 130 35.40620 -84.08142 3608 ocoee ocoee D 131 34.72990 -83.38580 4055 folkertsi folkertsi 132 34.66750 -83.31630 4007 quadramaculatus quadramaculatus A 132 34.66750 -83.31630 4007 quadramaculatus quadramaculatus A 132 34.66750 -83.31630 4008 monticola monticola B 133 34.67172 -83.31433 3603 ocoee ocoee D 133 34.67172 -83.31433 4078 quadramaculatus quadramaculatus A 134 34.69454 -83.32927 4024 ocoee ocoee FGH 135 35.34768 -83.97356 3585 aeneus 135 35.34768 -83.97356 3652 auriculatus auriculatus B 136 32.58800 -81.20197 3738 fuscus A 137 35.92794 -84.54158 3	3946 monticola monticola B 130 35.40620 -84.08142 TN 3947 monticola monticola B 130 35.40620 -84.08142 TN 3608 ocoee ocoee D 131 34.72990 -83.38580 GA 4055 folkertsi folkertsi 132 34.66750 -83.31630 GA 4007 quadramaculatus quadramaculatus A 132 34.66750 -83.31630 GA 4007 quadramaculatus quadramaculatus A 132 34.66750 -83.31630 GA 4008 monticola monticola B 133 34.67172 -83.31433 SC 3603 ocoee ocoee D 133 34.67172 -83.31433 SC 4078 quadramaculatus quadramaculatus A 134 34.69454 -83.2927 SC 4067 quadramaculatus quadramaculatus A 135 35.34768 -83.97356 NC 3652 auriculatus B 136

BEAMER

TABLE 1. (Continued)										
Specimen #	Genbank Accession #	Species	mtDNA clade	Locality	Latitude	Longitude	State	County		
DAB4919	MH403956	monticola	monticola B	142	35.25579	-83.80502	NC	Graham		
DAB4920	MH404023	ocoee	ocoee FGH	142	35.25579	-83.80502	NC	Graham		
DAB1804	MH404074	quadramaculatus	quadramaculatus A	143	34.66760	-83.20500	SC	Oconee		
DAB1805	MH403599	ocoee	ocoee D	143	34.66760	-83.20500	SC	Oconee		
DAB2017	MH403600	ocoee	ocoee D	143	34.66760	-83.20500	SC	Oconee		
DAB2024	MH403601	ocoee	ocoee D	143	34.66760	-83.20500	SC	Oconee		
DAB2042	MH403602	ocoee	ocoee D	143	34.66760	-83.20500	SC	Oconee		
DAB2503	MH403777	conanti	conanti A	144	33.52608	-82.00028	SC	Aiken		
DAB4775	MH404075	quadramaculatus	quadramaculatus A	145	34.88264	-83.35104	GA	Rabun		
DAB4779	MH403583	aeneus	aeneus	146	34.88218	-83.35019	GA	Rabun		
DAB3915	MH403562	wrighti	wrighti	147	35.06882	-83.53022	NC	Macon		
DAB3923	MH403582	aeneus	aeneus	147	35.06882	-83.53022	NC	Macon		
DAB3929	MH404019	ocoee	ocoee E	147	35.06882	-83.53022	NC	Macon		
DAB4024	MH404018	ocoee	ocoee E	148	35.08555	-83.52731	NC	Macon		
DAB4025	MH404070	quadramaculatus	quadramaculatus A	148	35.08555	-83.52731	NC	Macon		
DAB3947	MH404020	ocoee	ocoee E	149	35.15072	-83.58006	NC	Macon		
DAB4968	MH403948	monticola	monticola B	150	35.29718	-83.69929	NC	Graham		
DAB4577	MH403720	fuscus	fuscus B	151	38.08266	-86.47032	IN	Perry		
DAB4965	MH404014	ocoee	ocoee E	152	35.30923	-83.69466	NC	Graham		
DAB353	MH404017	ocoee	ocoee E	153	35.05396	-83.43548	NC	Macon		
DAB579	MH404069	quadramaculatus	quadramaculatus A	154	35.26449	-83.58300	NC	Macon		
DAB959	MH404047	marmoratus	quadramaculatus/mar-	154	35.26449	-83.58300	NC	Macon		
			moratus C							
DAB1122	MH404016	ocoee	ocoee E	155	35.26458	-83.58305	NC	Macon		
DAB3988	MH403958	monticola	monticola B	155	35.26458	-83.58305	NC	Macon		
DAB3989	MH404071	quadramaculatus	quadramaculatus A	155	35.26458	-83.58305	NC	Macon		
DAB251	MH403789	conanti	conanti A	156	33.13742	-81.43354	SC	Barnwell		
DAB440	MH404085	conanti	conanti A	156	33.13742	-81.43354	SC	Barnwell		
DAB2841	MH403780	conanti	conanti A	157	33.68164	-81.91269	SC	Edgefield		
DAB1291	MH403598	<i>осоее</i>	ocoee D	158	34.98040	-83.14770	GA	Rabun		

TABLE 1. (Continued)									
Specimen #	Genbank Accession #	Species	mtDNA clade	Locality	Latitude	Longitude	State	County	
DAB1336	MH404057	marmoratus	marmoratus B	158	34.98040	-83.14770	GA	Rabun	
DAB1337	MH404076	marmoratus	marmoratus B	158	34.98040	-83.14770	GA	Rabun	
DAB2237	MH403645	auriculatus	auriculatus B	159	33.05280	-81.10210	SC	Bamberg	
DAB2236	MH403653	auriculatus	auriculatus B	160	33.05011	-81.09828	SC	Bamberg	
DAB4928	MH404046	marmoratus	quadramaculatus/mar-	161	35.33470	-83.37220	NC	Swain	
			moratus C						
DAB4931	MH404077	quadramaculatus	quadramaculatus A	161	35.33470	-83.37220	NC	Swain	
DAB4733	MH403634	fuscus	fuscus C	162	33.32837	-81.30481	SC	Barnwell	
DAB4133	MH403806	santeetlah	santeetlah	163	35.68247	-83.63821	TN	Sevier	
DAB4134	MH404060	quadramaculatus	quadramaculatus F	163	35.68247	-83.63821	TN	Sevier	
DAB4135	MH403998	monticola	monticola B	163	35.68247	-83.63821	TN	Sevier	
DAB4729	MH403643	auriculatus	auriculatus B	164	32.61663	-80.56054	SC	Colleton	
DAB2787	MH403769	conanti	conanti A	165	33.76081	-81.69550	SC	Aiken	
DAB2810	MH403770	conanti	conanti A	166	33.63849	-81.56083	SC	Aiken	
DAB2890	MH403772	conanti	conanti A	167	33.55949	-81.48088	SC	Aiken	
DAB2795	MH403771	conanti	conanti A	168	33.47373	-81.37470	SC	Barnwell	
DAB2862	MH403635	fuscus	fuscus C	169	33.39159	-81.27278	SC	Barnwell	
DAB4058	MH404061	quadramaculatus	quadramaculatus F	170	35.63478	-83.49663	TN	Sevier	
DAB4059	MH403865	imitator	imitator	170	35.63478	-83.49663	TN	Sevier	
DAB4323	MH403651	auriculatus	auriculatus B	171	33.11908	-80.96501	SC	Bamberg	
DAB4104	MH403862	imitator	imitator	172	35.60979	-83.44936	TN	Sevier	
DAB4109	MH403866	imitator	imitator	172	35.60979	-83.44936	TN	Sevier	
DAB4110	MH403807	santeetlah	santeetlah	172	35.60979	-83.44936	TN	Sevier	
DAB4365	MH404062	quadramaculatus	quadramaculatus F	172	35.60979	-83.44936	NC	Sevier	
DAB500	MH403648	auriculatus	auriculatus B	173	32.89350	-80.68880	SC	Colleton	
DAB4760	MH403564	wrighti	wrighti	174	35.65361	-83.44222	NC	Sevier	
DAB4163	MH404059	quadramaculatus	quadramaculatus F	175	35.56747	-83.33715	NC	Swain	
DAB4164	MH403808	santeetlah	santeetlah	175	35.56747	-83.33715	NC	Swain	
DAB4165	MH403863	imitator	imitator	175	35.56747	-83.33715	NC	Swain	
DAB4167	MH403952	monticola	monticola B	175	35.56747	-83.33715	NC	Swain	
DAB4169	MH403809	santeetlah	santeetlah	176	35.56717	-83.33526	NC	Swain	

BEAMER

Specimen #	Genbank Accession #	Species	mtDNA clade	Locality	Latitude	Longitude	State	County
DAB881	MH403637	fuscus	fuscus C	177	33.38560	-81.13511	SC	Bamberg
DAB4949	MH404001	monticola	monticola B	178	35.40508	-83.14168	NC	Jackson
DAB2851	MH403636	fuscus	fuscus C	179	33.37898	-81.10430	SC	Orangeburg
DAB4415	MH403642	auriculatus	auriculatus B	180	32.69376	-80.38311	SC	Charleston
DAB4075	MH404086	santeetlah	santeetlah	181	35.51616	-83.19382	NC	Jackson
DAB4082	MH403864	imitator	imitator	181	35.51616	-83.19382	NC	Jackson
DAB3885	MH403778	conanti	conanti A	182	33.75139	-81.42895	SC	Aiken
DAB4152	MH403813	santeetlah	santeetlah	183	35.45904	-83.13078	NC	Jackson
DAB4945	MH403814	santeetlah	santeetlah	184	35.50186	-83.16920	NC	Jackson
DAB3978	MH403812	0 <i>c</i> 0 <i>e</i> e	ocoee A	185	35.47659	-83.13917	NC	Jackson
DAB3979	MH403613	0 <i>c</i> 0 <i>e</i> e	ocoee A	185	35.47659	-83.13917	NC	Jackson
DAB3980	MH403614	0 <i>c</i> 0 <i>e</i> e	ocoee A	185	35.47659	-83.13917	NC	Jackson
DAB3934	MH403612	0 <i>c</i> 0 <i>e</i> e	ocoee B	186	35.32178	-82.96523	NC	Jackson
DAB1823	MH403776	conanti	conanti A	187	34.00680	-81.62120	SC	Saluda
DAB1131	MH403811	santeetlah	santeetlah	188	35.43295	-83.00887	NC	Haywood
DAB1140	MH403961	monticola	monticola B	188	35.43295	-83.00887	NC	Haywood
DAB2687	MH404072	quadramaculatus	quadramaculatus A	189	35.35126	-82.90706	NC	Haywood
DAB2689	MH403611	<i>ocoee</i>	ocoee B	189	35.35126	-82.90706	NC	Haywood
DAB2878	MH403969	monticola	monticola B	189	35.35126	-82.90706	NC	Haywood
DAB4891	MH403782	conanti	conanti A	190	34.32113	-81.86235	SC	Laurens
DAB2902	MH404049	marmoratus	quadramaculatus/mar- moratus C	191	35.46794	-82.95703	NC	Haywood
DAB2903	MH403959	monticola	monticola B	191	35.46794	-82.95703	NC	Haywood
DAB2908	MH403970	monticola	monticola B	191	35.46794	-82.95703	NC	Haywood
DAB4757	MH403810	santeetlah	santeetlah	191	35.46794	-82.95703	NC	Haywood
DAB4768	MH403565	wrighti	wrighti	192	35.58471	-83.06228	NC	Haywood
DAB4910	MH403800	conanti	conanti A	193	35.26753	-82.72825	NC	Transylvania
DAB954	MH403996	monticola	monticola B	194	35.26765	-82.72826	NC	Transylvania
DAB958	MH404003	monticola	monticola B	195	35.29602	-82.74093	NC	Transylvania
DAB1808	MH403803	conanti	conanti A	196	34.92700	-82.36720	SC	Greenville
DAB4327	MH403779	conanti	conanti A	197	33.72368	-81.09925	SC	Lexington

TABLE 1. (Continued)

TABLE 1. (Continued)										
Specimen #	Genbank Accession #	Species	mtDNA clade	Locality	Latitude	Longitude	State	County		
DAB1813	MH403804	conanti	conanti A	198	35.00300	-82.33740	SC	Greenville		
DAB707	MH404048	quadramaculatus	quadramaculatus/mar-	199	35.73949	-83.01401	NC	Haywood		
			moratus C							
DAB713	MH403999	monticola	monticola B	199	35.73949	-83.01401	NC	Haywood		
DAB643	MH403950	monticola	monticola B	200	35.25990	-82.53150	NC	Henderson		
DAB646	MH403801	conanti	conanti A	200	35.25990	-82.53150	NC	Henderson		
DAB2122	MH403650	auriculatus	auriculatus B	201	32.95950	-80.20540	SC	Dorchester		
DAB1803	MH403872	fuscus	carolinensis	202	34.55450	-81.79960	SC	Laurens		
DAB1161	MH403868	carolinensis	carolinensis	203	35.68750	-82.89710	NC	Madison		
DAB1355	MH404050	quadramaculatus	quadramaculatus/mar-	203	35.68750	-82.89710	NC	Madison		
			moratus C							
DAB1800	MH404080	fuscus	fuscus C	204	33.19800	-80.33570	SC	Dorchester		
DAB2246	MH403638	fuscus	fuscus C	205	33.22220	-80.33310	SC	Berkeley		
DAB2247	MH403639	fuscus	fuscus C	205	33.22220	-80.33310	SC	Berkeley		
DAB2234	MH403875	fuscus	carolinensis	206	34.05045	-81.15124	SC	Lexington		
DAB2254	MH403903	fuscus	carolinensis	207	34.18459	-81.27431	SC	Richland		
DAB4869	MH403906	fuscus	carolinensis	208	34.40005	-81.47656	SC	Newberry		
DAB3851	MH403914	fuscus	carolinensis	209	34.01208	-81.08300	SC	Lexington		
DAB1801	MH403900	fuscus	carolinensis	210	34.00920	-81.07390	SC	Richland		
DAB3854	MH403898	fuscus	carolinensis	210	34.00920	-81.07390	SC	Lexington		
DAB4825	MH403913	fuscus	carolinensis	211	34.40722	-81.42715	SC	Newberry		
DAB1723	MH403632	fuscus	fuscus C	212	33.70060	-80.71610	SC	Calhoun		
DAB1751	MH403633	fuscus	fuscus C	212	33.70060	-80.71610	SC	Calhoun		
DAB1770	MH403871	fuscus	carolinensis	213	33.82978	-80.82590	SC	Richland		
DAB350	MH403911	fuscus	carolinensis	214	33.74340	-80.73460	SC	Calhoun		
DAB4311	MH403802	conanti	conanti A	215	34.9344	-81.91146	SC	Spartanburg		
DAB1761	MH403631	fuscus	fuscus C	216	33.70574	-80.64707	SC	Calhoun		
DAB1272	MH403622	welteri	welteri	217	36.66770	-83.58262	KY	Bell		
DAB1105	MH403873	carolinensis	carolinensis	218	35.46161	-82.36671	NC	Buncombe		
DAB1080	MH404029	quadramaculatus	quadramaculatus/mar-	219	35.47187	-82.36341	NC	Henderson		
			moratus E							

16 · Zootaxa 4734 (1) © 2020 Magnolia Press

BEAMER

Specimen #	Genbank Accession #	Species	mtDNA clade	Locality	Latitude	Longitude	State	County
DAB1112	MH403964	monticola	monticola B	219	35.47187	-82.36341	NC	Henderson
DAB1144	MH404052	marmoratus	quadramaculatus/mar-	219	35.47187	-82.36341	NC	Henderson
			moratus C					
DAB2821	MH403870	carolinensis	carolinensis	220	35.79749	-82.66362	NC	Madison
DAB2822	MH403979	monticola	monticola B	220	35.79749	-82.66362	NC	Madison
AB3507	MH403874	carolinensis	carolinensis	221	35.46345	-82.32281	NC	Henderson
AB1818	MH403910	fuscus	carolinensis	222	34.61180	-81.43760	SC	Union
AB1036	MH403702	fuscus	fuscus B	223	38.72338	-85.45903	IN	Jefferson
AB1799	MH403896	fuscus	carolinensis	224	33.80550	-80.53520	SC	Sumter
AB2055	MH403912	fuscus	carolinensis	224	33.80550	-80.53520	SC	Sumter
AB4952	MH403879	carolinensis	carolinensis	225	35.70910	-82.39394	NC	Buncombe
AB1275	MH403869	carolinensis	carolinensis	226	35.98116	-82.66407	NC	Madison
AB1356	MH404051	quadramaculatus	quadramaculatus/mar-	226	35.98116	-82.66407	NC	Madison
			moratus C					
AB1360	MH403955	monticola	monticola B	226	35.98116	-82.66407	NC	Madison
AB945	MH403566	organi	organi	227	35.76481	-82.26512	NC	Yancey
AB946	MH403880	carolinensis	carolinensis	227	35.76481	-82.26512	NC	Yancey
AB1617	MH403752	ochrophaeus	ochrophaeus	228	36.93660	-83.37300	KY	Leslie
DAB1627	MH403967	monticola	monticola B	228	36.93660	-83.37300	KY	Leslie
AB2561	MH403644	auriculatus	auriculatus B	229	33.46256	-79.89099	SC	Williamsburg
DAB942	MH404031	quadramaculatus	quadramaculatus/mar-	230	35.75372	-82.14808	NC	McDowell
			moratus E					
DAB943	MH403876	carolinensis	carolinensis	230	35.75372	-82.14808	NC	McDowell
DAB2672	MH404025	quadramaculatus	quadramaculatus/mar-	231	35.92322	-82.26500	NC	Yancey
	N 41 40 20 50	<i>1</i>	moratus E	001	25.02222	00.04500		
AB2676	MH403878	carolinensis	carolinensis	231	35.92322	-82.26500	NC	Yancey
AB2/10	MH403904	carolinensis	carolinensis	232	35.82968	-82.10637	NC	McDowell
AB2719	MH403957	monticola	monticola B	232	35.82968	-82.10637	NC	McDowell
AB2724	MH404030	quadramaculatus	quadramaculatus/mar-	232	35.82968	-82.10637	NC	McDowell
AD(27	MIL402646		moratus E	222	22 22150	70 50020	50	Doubolory
AD03/	1111403040	auricululus	auriculatus D	233	33.23130	-/9.30030	30	Derkeley

TABLE 1. (Continued)									
Specimen #	Genbank Accession #	Species	mtDNA clade	Locality	Latitude	Longitude	State	County	
DAB1701	MH403621	welteri	welteri	234	36.77700	-83.01600	VA	Lee	
DAB1644	MH403753	ochrophaeus	ochrophaeus	235	36.94800	-83.18200	KY	Harlan	
DAB1649	MH403623	welteri	welteri	235	36.94800	-83.18200	KY	Harlan	
DAB2025	MH403908	fuscus	carolinensis	236	35.81230	-82.03460	NC	McDowell	
DAB1819	MH403625	fuscus	fuscus C	237	35.13460	-81.35600	SC	York	
DAB1676	MH403717	fuscus	fuscus B	238	37.24100	-83.39300	KY	Leslie	
DAB1683	MH404005	monticola	monticola B	238	37.24100	-83.39300	KY	Leslie	
DAB1273	MH403755	ochrophaeus	ochrophaeus	239	36.76339	-82.87937	VA	Lee	
DAB719	MH403749	orestes	orestes A	240	35.64120	-81.72290	NC	Burke	
DAB356	MH403897	fuscus	carolinensis	241	35.66035	-81.69752	NC	Burke	
DAB408	MH403971	monticola	monticola B	241	35.66035	-81.69752	NC	Burke	
DAB751	MH403954	monticola	monticola B	241	35.66035	-81.69752	NC	Burke	
DAB755	MH403907	fuscus	carolinensis	241	35.66035	-81.69752	NC	Burke	
DAB2690	MH403563	organi	organi	242	36.10411	-82.13355	NC	Mitchell	
DAB2693	MH403700	organi	organi	242	36.10411	-82.13355	NC	Mitchell	
DAB2762	MH403759	orestes	orestes B	242	36.10411	-82.13355	NC	Mitchell	
DAB2355	MH403629	fuscus	fuscus C	243	34.19649	-80.20240	SC	Lee	
DAB1711	MH403739	fuscus	fuscus A	244	36.70100	-82.69900	VA	Scott	
DAB1814	MH403628	fuscus	fuscus C	245	34.20830	-80.20000	SC	Lee	
DAB2698	MH404035	quadramaculatus	quadramaculatus/mar-	246	36.10186	-82.09199	NC	Mitchell	
			moratus E						
DAB2893	MH403760	orestes	orestes B	246	36.10186	-82.09199	NC	Mitchell	
DAB1172	MH403951	monticola	monticola B	247	35.92240	-81.90650	NC	Burke	
DAB1601	MH403754	ochrophaeus	ochrophaeus	248	36.91400	-82.89400	KY	Harlan	
DAB224	MH403624	welteri	welteri	249	36.94900	-82.87500	KY	Harlan	
DAB3420	MH404053	quadramaculatus	quadramaculatus/mar-	250	36.31749	-82.24348	TN	Carter	
			moratus C						
DAB3426	MH403877	fuscus	carolinensis	250	36.31749	-82.24348	TN	Carter	
DAB4320	MH403909	fuscus	carolinensis	251	34.66302	-80.52495	SC	Lancaster	
DAB3880	MH403641	auriculatus	auriculatus B	252	33.84032	-79.68609	SC	Florence	

TABLE 1. (Continued)										
Specimen #	Genbank Accession #	Species	pecies mtDNA clade Loca		Latitude	Longitude	State	County		
DAB534	MH404032	quadramaculatus	quadramaculatus/marmoratus E	253	36.1308	-81.97440	NC	Avery		
DAB1688	MH403711	fuscus	fuscus B	254	36.64900	-82.49130	VA	Scott		
DAB1694	MH403974	monticola	monticola B	254	36.64900	-82.49130	VA	Scott		
DAB1698	MH403978	monticola	monticola B	254	36.64900	-82.49130	VA	Scott		
DAB3494	MH403626	fuscus	fuscus C	255	35.74120	-81.56621	NC	Burke		
DAB2046	MH403762	orestes	orestes B	256	36.11025	-81.88097	NC	Avery		
DAB3658	MH403678	fuscus	fuscus B	257	36.53049	-82.29366	TN	Sullivan		
DAB1809	MH403901	fuscus	carolinensis	258	36.01350	-81.68990	NC	Caldwell		
DAB1810	MH403748	orestes	orestes A	258	36.01350	-81.68990	NC	Caldwell		
DAB1811	MH404009	monticola	monticola B	258	36.01350	-81.68990	NC	Caldwell		
DAB1812	MH404033	quadramaculatus	quadramaculatus/marmoratus E	258	36.01350	-81.68990	NC	Caldwell		
DAB411	MH404028	quadramaculatus	quadramaculatus/marmoratus E	259	36.11420	-81.77860	NC	Caldwell		
DAB660	MH404034	quadramaculatus	quadramaculatus/marmoratus E	259	36.11420	-81.77860	NC	Caldwell		
DAB700	MH404027	marmoratus	quadramaculatus/marmoratus E	259	36.11420	-81.77860	NC	Caldwell		
DAB739	MH403761	orestes	orestes B	259	36.11420	-81.77860	NC	Caldwell		
DAB4315	MH403630	fuscus	fuscus C	260	34.60717	-80.24472	SC	Chesterfield		
DAB2873	MH403627	fuscus	fuscus C	261	34.30910	-79.92905	SC	Darlington		
DAB2006	MH403902	fuscus	carolinensis	262	36.08670	-81.69030	NC	Caldwell		
DAB352	MH403915	fuscus	carolinensis	263	33.99555	-79.51902	SC	Florence		
DAB806	MH403916	fuscus	carolinensis	263	33.99555	-79.51902	SC	Florence		
DAB2028	MH403905	fuscus	carolinensis	264	35.14173	-80.64418	NC	Mecklenburg		
DAB2043	MH403675	fuscus	fuscus B	265	36.20858	-81.68937	NC	Watauga		
DAB2044	MH403763	fuscus	fuscus B	265	36.20858	-81.68937	NC	Watauga		
DAB3557	MH403984	monticola	monticola B	266	36.55706	-82.01443	TN	Sullivan		
DAB3559	MH403672	fuscus	fuscus B	266	36.55706	-82.01443	TN	Sullivan		
DAB524	MH403965	monticola	monticola B	267	36.22360	-81.66620	NC	Watauga		
DAB526	MH403676	fuscus	fuscus B	267	36.22360	-81.66620	NC	Watauga		
DAB531	MH404040	quadramaculatus	quadramaculatus/marmoratus E	267	36.22360	-81.66620	NC	Watauga		
DAB1894	MH404026	quadramaculatus	quadramaculatus/marmoratus E	268	36.15120	-81.58240	NC	Watauga		
DAB1904	MH403747	orestes	orestes A	268	36.15120	-81.58240	NC	Watauga		

TABLE 1. (Continued)									
Specimen #	pecimen # Genbank Accession # Species mtDNA clade		mtDNA clade	Locality	Latitude	Longitude	State	County	
DAB3515	MH403972	monticola	monticola B	269	36.13524	-81.55829	NC	Watauga	
DAB3516	MH403746	orestes	orestes A	269	36.13524	-81.55829	NC	Watauga	
DAB3518	MH403882	fuscus	carolinensis	269	36.13524	-81.55829	NC	Watauga	
DAB3675	MH403673	fuscus	fuscus B	270	36.53876	-81.94912	TN	Sullivan	
DAB3676	MH404089	monticola	monticola B	270	36.53876	-81.94912	TN	Sullivan	
DAB2264	MH403726	auriculatus	auriculatus C	271	34.32643	-79.72534	SC	Darlington	
DAB715	MH403883	fuscus	carolinensis	272	35.95873	-81.33990	NC	Caldwell	
DAB1925	MH404039	quadramaculatus	quadramaculatus/marmoratus E	273	36.21689	-81.56841	NC	Watauga	
DAB2014	MH403968	monticola	monticola B	273	36.21689	-81.56841	NC	Watauga	
DAB2027	MH403881	fuscus	carolinensis	274	36.16760	-81.50250	NC	Watauga	
DAB996	MH403719	fuscus	fuscus B	275	38.08065	-83.40957	KY	Bath	
DAB1039	MH403718	fuscus	fuscus B	275	38.08065	-83.40957	KY	Bath	
DAB4699	MH404082	auriculatus	auriculatus C	276	33.76596	-79.03564	SC	Horry	
DAB4430	MH403724	auriculatus	auriculatus C	277	34.48521	-79.67188	SC	Marlboro	
DAB4786	MH403659	fuscus	fuscus D	278	34.78050	-79.90467	SC	Marlboro	
DAB1516	MH404041	quadramaculatus	quadramaculatus/marmoratus E	279	36.09520	-81.08887	NC	Wilkes	
DAB1517	MH403885	fuscus	carolinensis	279	36.09520	-81.08887	NC	Wilkes	
DAB3100	MH403567	organi	organi	280	36.63881	-81.61039	VA	Smyth	
DAB3500	MH403568	organi	organi	280	36.63881	-81.61039	VA	Smyth	
DAB3170	MH403997	monticola	monticola B	281	36.61499	-81.56426	VA	Grayson	
DAB3171	MH404038	quadramaculatus	quadramaculatus/marmoratus E	281	36.61499	-81.56426	VA	Grayson	
DAB3156	MH403674	fuscus	fuscus B	282	36.64793	-81.58443	VA	Smyth	
DAB3585	MH403671	fuscus	fuscus B	282	36.64793	-81.58443	VA	Smyth	
DAB3586	MH403765	orestes	orestes C	282	36.64793	-81.58443	VA	Smyth	
DAB3346	MH404044	quadramaculatus	quadramaculatus/marmoratus E	283	36.65481	-81.58453	VA	Smyth	
DAB3264	MH403764	orestes	orestes C	284	36.61133	-81.48774	VA	Grayson	
DAB3272	MH404013	monticola	monticola B	284	36.61133	-81.48774	VA	Grayson	
DAB3275	MH404037	quadramaculatus	quadramaculatus/marmoratus E	284	36.61133	-81.48774	VA	Grayson	
DAB4420	MH403721	auriculatus	auriculatus C	285	34.57243	-79.43999	SC	Dillon	
DAB1477	MH403884	fuscus	fuscus D	286	36.03724	-80.89486	NC	Iredell	

TABLE 1. (Continued)									
Specimen #	Genbank Accession #	Species	mtDNA clade	Locality	Latitude	Longitude	State	County	
DAB1506	MH403886	fuscus	fuscus D	286	36.03724	-80.89486	NC	Iredell	
DAB1488	MH403656	fuscus	fuscus D	287	35.20924	-79.83497	NC	Montgomery	
DAB638	MH403660	fuscus	fuscus D	288	35.42695	-79.99377	NC	Montgomery	
DAB1496	MH403663	fuscus	fuscus D	288	35.42695	-79.99377	NC	Montgomery	
DAB1484	MH403654	fuscus	fuscus D	289	35.94446	-80.48492	NC	Davie	
DAB437	MH403661	fuscus	fuscus D	290	34.99971	-79.51694	NC	Scotland	
DAB780	MH403664	fuscus	fuscus D	290	34.99971	-79.51694	NC	Scotland	
DAB782	MH403658	fuscus	fuscus D	291	34.99000	-79.49220	NC	Scotland	
DAB3298	MH403679	fuscus	fuscus B	292	36.63301	-81.08066	VA	Grayson	
DAB1505	MH403657	fuscus	fuscus D	293	35.78419	-80.20717	NC	Davidson	
DAB3400	MH403723	fuscus	fuscus E	294	36.61492	-80.77537	VA	Carroll	
DAB3260	MH403722	fuscus	auriculatus C	295	36.61210	-80.77138	VA	Carroll	
DAB3262	MH404043	quadramaculatus	quadramaculatus/marmoratus E	295	36.61210	-80.77138	VA	Carroll	
DAB1485	MH403725	auriculatus	auriculatus C	296	34.47120	-78.62880	NC	Bladen	
DAB1955	MH404000	monticola	monticola B	297	36.36080	-80.38670	NC	Stokes	
DAB1956	MH403949	monticola	monticola B	297	36.36080	-80.38670	NC	Stokes	
DAB1959	MH404042	quadramaculatus	quadramaculatus/marmoratus E	297	36.36080	-80.38670	NC	Stokes	
DAB2035	MH403888	fuscus	carolinensis	297	36.36080	-80.38670	NC	Stokes	
DAB2041	MH403985	monticola	monticola B	297	36.36080	-80.38670	NC	Stokes	
DAB1042	MH403692	fuscus	fuscus B	298	37.16570	-81.14673	VA	Bland	
DAB508	MH403665	fuscus	fuscus D	299	34.63030	-78.60630	NC	Bladen	
DAB2280	MH403887	fuscus	carolinensis	300	36.38920	-80.22490	NC	Stokes	
DAB2281	MH404036	quadramaculatus	quadramaculatus/marmoratus E	300	36.38920	-80.22490	NC	Stokes	
DAB2275	MH403894	fuscus	carolinensis	301	36.24310	-80.06590	NC	Forsyth	
DAB2278	MH403893	fuscus	carolinensis	302	36.31700	-80.13120	NC	Stokes	
DAB1545	MH403733	auriculatus	auriculatus C	303	34.18090	-77.94840	NC	New Hanover	
DAB3983	MH403620	planiceps	planiceps	304	36.69872	-80.44657	VA	Patrick	
DAB3985	MH403987	monticola	monticola B	304	36.69872	-80.44657	VA	Patrick	
DAB2277	MH403892	fuscus	carolinensis	305	36.16310	-79.88000	NC	Guilford	
DAB1983	MH403995	monticola	monticola B	306	36.72540	-80.38180	VA	Patrick	

TABLE 1. (Co	ntinued)							
Specimen #	Genbank Accession #	Species	mtDNA clade	Locality	Latitude	Longitude	State	County
DAB3649	MH404006	monticola	monticola B	307	38.13346	-81.68757	WV	Boone
DAB3599	MH403693	fuscus	fuscus B	308	38.18952	-81.62327	WV	Kanawha
DAB2276	MH403891	fuscus	carolinensis	309	36.17000	-79.51060	NC	Alamance
DAB3427	MH403750	ochrophaeus	ochrophaeus	310	37.42250	-80.50522	VA	Giles
DAB3379	MH404011	monticola	monticola B	311	37.43269	-80.51239	VA	Giles
DAB3381	MH403677	fuscus	fuscus B	311	37.43269	-80.51239	VA	Giles
DAB3476	MH404004	monticola	monticola B	312	37.43110	-80.49201	VA	Giles
DAB3477	MH403680	fuscus	fuscus B	312	37.43110	-80.49201	VA	Giles
DAB3695	MH403703	fuscus	fuscus B	313	37.44836	-80.49348	WV	Monroe
DAB3560	MH404090	quadramaculatus	quadramaculatus/marmoratus E	314	37.44841	-80.49314	WV	Monroe
DAB3561	MH403975	monticola	monticola B	314	37.44841	-80.49314	WV	Monroe
DAB3240	MH403988	monticola	monticola B	315	37.19186	-80.21007	VA	Montgomery
DAB3254	MH403983	monticola	monticola B	315	37.19186	-80.21007	VA	Montgomery
DAB3257	MH403973	monticola	monticola B	315	37.19186	-80.21007	VA	Montgomery
DAB75159	MH403666	fuscus	fuscus D	316	35.67630	-78.6362	NC	Wake
DAB3522	MH404010	monticola	monticola B	317	37.18289	-80.13977	VA	Roanoke
DAB3532	MH403712	fuscus	fuscus B	317	37.18289	-80.13977	VA	Roanoke
DAB3512	MH403981	monticola	monticola B	318	37.1546	-80.09840	VA	Roanoke
DAB1478	MH403668	fuscus	fuscus D	319	35.02620	-77.94430	NC	Duplin
DAB2279	MH403895	fuscus	carolinensis	320	36.30610	-79.21950	NC	Caswell
DAB3398	MH403619	planiceps	planiceps	321	37.18173	-79.95617	VA	Roanoke
DAB3693	MH403690	fuscus	fuscus B	322	38.81141	-81.51319	WV	Roane
DAB2411	MH403662	fuscus	fuscus D	323	36.31166	-78.74913	NC	Granville
DAB3554	MH403710	fuscus	fuscus B	324	37.73963	-80.03075	VA	Alleghany
DAB3302	MH403962	monticola	monticola B	325	37.85277	-80.12182	VA	Alleghany
DAB3317	MH403953	monticola	monticola B	326	37.89437	-80.10871	VA	Alleghany
DAB3318	MH403705	fuscus	fuscus B	326	37.89437	-80.10871	VA	Alleghany
DAB3187	MH403709	fuscus	fuscus B	327	37.55162	-79.74302	VA	Botetourt
DAB3648	MH403966	monticola	monticola B	328	37.70380	-79.89080	VA	Botetourt
DAB3338	MH403963	monticola	monticola B	329	37.48810	-79.66629	VA	Botetourt

Specimen #	Genbank Accession #	Species	mtDNA clade	Locality	Latitude	Longitude	State	County
DAB1487	MH403735	auriculatus	auriculatus C	330	34.76809	-76.94343	NC	Carteret
DAB3195	MH403989	monticola	monticola B	331	37.47920	-79.64114	VA	Botetourt
DAB3201	MH403691	fuscus	fuscus B	331	37.47920	-79.64114	VA	Botetourt
DAB3631	MH403982	monticola	monticola B	332	37.69391	-79.79707	VA	Botetourt
DAB3633	MH403704	fuscus	fuscus B	332	37.69391	-79.79707	VA	Botetourt
DAB3286	MH403960	monticola	monticola B	333	37.48124	-79.52765	VA	Bedford
DAB3290	MH403688	fuscus	fuscus B	333	37.48124	-79.52765	VA	Bedford
DAB3184	MH403990	monticola	monticola B	334	37.49452	-79.53653	VA	Bedford
DAB2413	MH403667	fuscus	fuscus D	335	36.29792	-78.28969	NC	Vance
DAB414	MH403732	auriculatus	auriculatus C	336	34.98179	-76.95098	NC	Craven
DAB3179	MH403707	fuscus	fuscus B	337	37.71338	-79.67126	VA	Rockbridge
DAB3181	MH403986	monticola	monticola B	337	37.71338	-79.67126	VA	Rockbridge
DAB2956	MH403655	fuscus	fuscus D	338	36.62038	-78.56461	VA	Mecklenburg
DAB3644	MH403757	ochrophaeus	ochrophaeus	339	38.19283	-80.13548	WV	Pocahontas
DAB434	MH403734	auriculatus	auriculatus C	340	35.75819	-77.69613	NC	Edgecombe
DAB209	MH403731	auriculatus	auriculatus C	341	35.18479	-77.08233	NC	Craven
DAB972	MH403728	auriculatus	auriculatus C	342	35.71730	-77.53535	NC	Pitt
DAB290	MH403889	fuscus	carolinensis	343	35.71570	-77.51766	NC	Pitt
DAB441	MH403890	fuscus	carolinensis	343	35.71570	-77.51766	NC	Pitt
DAB3623	MH403685	fuscus	fuscus B	344	38.83992	-80.60684	WV	Braxton
DAB201	MH403729	auriculatus	auriculatus C	345	35.42873	-77.18937	NC	Beaufort
DAB2464	MH403727	auriculatus	auriculatus C	346	35.60175	-77.36124	NC	Pitt
DAB2992	MH403669	fuscus	fuscus D	347	36.91219	-78.39887	VA	Lunenburg
DAB3074	MH403689	fuscus	fuscus B	348	37.50837	-78.93576	VA	Amherst
DAB3601	MH404083	ochrophaeus	ochrophaeus	349	38.67958	-80.00866	WV	Randolph
DAB3602	MH403976	monticola	monticola B	349	38.67958	-80.00866	WV	Randolph
DAB3053	MH403694	fuscus	fuscus B	350	37.84382	-79.13306	VA	Nelson
DAB4097	MH403730	auriculatus	auriculatus C	351	35.88587	-77.12276	NC	Martin
DAB3081	MH403992	monticola	monticola B	352	37.86608	-79.02574	VA	Nelson
DAB1059	MH403980	monticola	monticola B	353	37.91813	-79.00668	VA	Augusta

TABLE 1. (Continued)

Specimen #	Genbank Accession #	Species	mtDNA clade	Locality	Latitude	Longitude	State	County
DAB3039	MH403670	fuscus	fuscus D	354	37.96298	-78.96586	VA	Augusta
DAB4086	MH403867	fuscus	carolinensis	355	36.72129	-77.59063	VA	Greensville
DAB602	MH403756	ochrophaeus	ochrophaeus	356	38.76740	-79.56230	WV	Randolph
DAB603	MH403683	fuscus	fuscus B	356	38.76740	-79.56230	WV	Randolph
DAB566	MH404012	monticola	monticola B	357	38.91306	-79.68684	WV	Randolph
DAB3413	MH404084	ochrophaeus	ochrophaeus	358	38.92986	-79.67971	WV	Randolph
DAB3618	MH403681	fuscus	fuscus B	359	38.01092	-78.70030	VA	Albemarle
DAB3534	MH403977	monticola	monticola B	360	38.08436	-78.72977	VA	Albemarle
DAB3552	MH403697	fuscus	fuscus B	360	38.08436	-78.72977	VA	Albemarle
DAB549	MH403714	fuscus	fuscus B	361	38.54269	-79.06630	VA	Rockingham
DAB596	MH403715	fuscus	fuscus B	362	38.66190	-79.08740	WV	Rockingham
DAB3014	MH403713	fuscus	fuscus B	363	37.85282	-77.89612	VA	Louisa
DAB4087	MH403716	fuscus	fuscus B	364	37.54002	-77.56281	VA	Richmond City
DAB2963	MH404081	fuscus	fuscus B	365	38.10458	-77.99510	VA	Louisa
DAB4045	MH403698	fuscus	fuscus B	366	37.70231	-77.38098	VA	Hanover
DAB3099	MH403708	fuscus	fuscus B	367	38.80439	-78.45050	VA	Shenandoah
DAB3023	MH403991	monticola	monticola B	368	38.68898	-78.33460	VA	Page
DAB3001	MH403696	fuscus	fuscus B	369	38.65388	-78.27149	VA	Rappahannock
DAB3588	MH403701	fuscus	fuscus B	370	37.14048	-76.73075	VA	Surry
DAB3067	MH403993	monticola	monticola B	371	38.61455	-78.15498	VA	Rappahannock
DAB2941	MH403695	fuscus	fuscus B	372	38.89657	-78.40739	VA	Shenandoah
DAB2975	MH403706	fuscus	fuscus B	373	37.20829	-76.57054	VA	Newport News
DAB3090	MH403699	fuscus	fuscus B	374	38.86463	-77.70308	VA	Fauquier
DAB1999	MH403751	ochrophaeus	ochrophaeus	375	41.74641	-79.17482	PA	Warren
DAB2000	MH403687	fuscus	fuscus B	375	41.74641	-79.17482	PA	Warren
DAB2051	MH403682	fuscus	fuscus B	375	41.74641	-79.17482	PA	Warren
DAB1988	MH403686	ochrophaeus	ochrophaeus	376	41.37141	-77.93276	PA	Clinton
DAB1996	MH403758	ochrophaeus	ochrophaeus	377	41.77378	-77.82801	PA	Potter
DAB1881	MH403684	fuscus	fuscus B	378	43.04236	-73.81226	NY	Saratoga
DAB4040	MH403899	fuscus	carolinensis	379			NC	

Sequence fragments were assembled in Geneious R6.1 (Kearse *et al.* 2012), corrected manually to produce a consensus sequence for each sample, for which coding regions were translated to ensure an appropriate reading frame. Consensus sequences were aligned using MUSCLE 3.6 (Edgar 2004) and any positions for which homology could not be unambiguously established were excluded. *Phaeognathus hubrichti*, the sister taxon of *Desmognathus*, served as an outgroup in phylogenetic analyses.

Phylogenetic analyses. Phylogenies were generated using Bayesian inference implemented with the software MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). We partitioned the dataset by gene and codon position and used MrModeltest 2.0 (Nylander 2004) to identify appropriate models of DNA substitution by Akaike Information Criterion. The analysis consisted of two concurrent runs with four simultaneous chains and a sample frequency of 1000. To ensure convergence on the same topology, we allowed each analysis to run until the split standard deviation was < 0.01 and the PSRF value was near 1. Many additional replicate analyses with the same parameters were conducted to ensure that chain mixing was adequate and that the analysis was not trapped on local optima. Trees sampled prior to convergence were discarded, and the remaining trees were used to calculate posterior probabilities. Likelihood scores were calculated for all post-convergence topologies using the Sump command in MrBayes.

A Maximum Likelihood (ML) reconstruction was implemented in GARLI (Bazinet *et al.* 2014). We again used MrModeltest 2.0 (Nylander 2004) to identify an appropriate model of DNA substitution by Akaike Information Criterion for the unpartitioned dataset. Nodal support was estimated via 100 bootstrap replicates.

Delimitation of reciprocally monophyletic clades. Our dataset included all recognized species as well as most of the independent evolutionary clades identified in Kozak *et al.* (2005b), which were confirmed as a function of gene sequence overlap. Kozak *et al.* (2005b) used the tree-based method of Wiens and Penkrot (2002) to delimit clades, which in turn informed our delimitation of reciprocally monophyletic clades here. We chose to maximize the genetic diversity and geographic area sampled at the expense of within population sampling (Lemmon *et al.* 2007) and, in general, collected mtDNA sequence data from a single individual for most populations. This approach makes it impossible to identify gene flow by determining whether haplotypes are shared between populations. As a result we have only identified additional mtDNA clades that were genealogically exclusive and displayed concordance with geography. Clade nomenclature follows (and complements) the naming protocol of Kozak *et al.* (2005b), where a clade is designated by a valid species name (e.g., *Desmognathus wrighti* King) or specific epithet/capital letter combination (e.g., *fuscus* A).

Results and discussion

Phylogenetic analysis. The mtDNA sequence dataset included 536 species X site samples, yielding 1,926 aligned bases (all length variable regions were excluded) and a total of 530 unique haplotypes (Table 1). Models of DNA substitution estimated for the partitions were: *ND2* 1st position (GTR+I+G), *ND2* 2nd (F81), *ND2* 3rd (GTR+I+G), tRNA's (F81), *COX1* 1st position (GTR+I+G), *COX1* 2nd (GTR+I+G). Convergence occurred at 30,000,000 generations (300,000 trees sampled prior to convergence were discarded). The Bayesian analysis produced a majority-rule consensus tree with a mean log likelihood of -53051.94. A collapsed consensus tree is shown in Fig. 3.

A single model of nucleotide evolution, GTR+I+G was estimated for the alignment and utilized for the ML point estimate. The topologies of the Bayesian and ML reconstructions were very similar and can be summarized as follows: the bootstrapped ML reconstruction (Fig. 4) contained several polytomies in sections of the topology that have low posterior probabilities in the Bayesian reconstruction. A single point estimate ML reconstruction is nearly identical to the Bayesian topology, with the following exceptions: 1) *Desmognathus aeneus* Brown and Bishop and *D. imitator* Dunn are depicted as sister species and 2) a clade comprising *D. quadramaculatus* Holbrook, *D. marmoratus* Moore and *D. folkertsi* Camp, Tilley, Austin, and Marshall branches before *D. aeneus* and *D. imitator*. Moreover, Bayesian and ML topologies are very similar to that presented by Kozak *et al.* (2005b), with one notable exception in which our reconstructions depict a sister relationship between *D. brimleyorum* Stejneger and a clade composed primarily of populations of *D. conanti* Rossman. Due to the similarity in topology between the single point estimate ML reconstruction and the Bayesian ML reconstruction, only the bootstrapped ML reconstruction is presented here (Fig. 4).



0 05

FIGURE 3. Bayesian majority-rule consensus phylogram. Genealogically exclusive clades are collapsed and labeled to match those in Kozak *et al.* 2005b. Previously unsampled genealogically exclusive clades have been given the next available letter. Posterior probabilities greater than 0.90 are indicated by diamonds.

Delimitation of reciprocally monophyletic clades. We recovered a total of 45 reciprocally monophyletic clades, 34 of which correspond to the 35 clades identified by Kozak *et al.* (2005b), the most comprehensive phylogenetic reconstruction of *Desmognathus* published to date. Because we only sampled a single individual per species at each site, thus violating the assumptions of the Wiens and Penkrot (2002) method, we have only delineated genealogically exclusive clades not sampled by Kozak *et al.* (2005). We do not count their *Desmognathus auriculatus* Holbrook lineage as being separate from their *conanti* C clade (Beamer & Lamb 2008), so their lineage scheme is reduced to 34 for comparisons herein. Only one of our additional clades represent a clade from Kozak *et al.*'s (2005b) alternative 44 clade scheme. Our remaining 12 clades were not sampled in their study, and two of these clades have not been sampled in any previous molecular phylogenetic survey.



FIGURE 4. Maximum-likelihood reconstruction, numbers above branches represent bootstrap support values (only bootstrap values below 100% are shown), clades are labeled as in Figure 3.

mtDNA clades. In this section we outline the phylogenetic placement, relationships, and geographic distribution for each of the 45 reciprocally monophyletic clades recovered in the Bayesian and ML reconstructions. The general order of clade accounts corresponds to clade placement, from basal to more derived clades, in the Bayesian tree. As noted, clades bear names in accord with current taxonomy, and as such, narrowly distributed species are often represented by just one clade whereas more widely-distributed species may be composed of multiple clades. In some cases, clade relationships have resulted in specific epithets occupying disparate topological positions across the tree and/or falling outside traditionally recognized species complexes. Ecoregion X drainage population samples are depicted for each clade, and those clades whose geographic distributions differ significantly from previous accounts are noted.

1-2. Desmognathus wrighti and D. organi

Our phylogenetic reconstructions recovered a clade comprising two genealogically exclusive clades of pygmy salamanders, *Desmognathus wrighti* and *D. organi* Crespi, Browne, and Rissler, which are sister to all remaining *Desmognathus* clades (Fig. 3). The sister relationship between these two species/clades has been recovered previously (Crespi *et al.* 2010; Kozak *et al.* 2005b), as has the sister relationship of pygmy salamanders to the other dusky salamanders (Chippindale *et al.* 2004; Kozak *et al.* 2005b; Mueller *et al.* 2004; Rissler & Taylor 2003; Titus & Lar-

son 1996). One of the clades contains three populations that correspond to *D. wrighti* as ascertained via a topotypic sample (pop. 174), and the second clade, also represented by three populations, corresponds to *D. organi*, which is also represented by topotypic material (pop. 280; Fig. 5A).

Distribution. The distribution of these two sister species as sampled herein (Fig. 6) does not differ from the previously described range (Crespi *et al.* 2010).



FIGURE 5. Branches pruned from Bayesian majority-rule consensus phylogram, diamonds represent posterior probabilities > 0.90. Numbers following species names in parenthesis represent population sample numbers. A. Branch containing *Desmognathus organi* and *D. wrighti*. B. Branch containing *Desmognathus imitator*. C. Branch containing *Desmognathus aeneus*.





FIGURE 6. Sampling localities for Desmognathus organi and D. wrighti, symbols match those in Fig. 5A.



FIGURE 7. Sampling localities for *Desmognathus imitator* and *D. aeneus*, symbols match those in Fig. 5B and 5C respectively.

Alabama

3. Desmognathus imitator

Our Bayesian and ML reconstructions recovered a genealogically exclusive clade comprising four populations of *Desmognathus imitator*. This lineage includes topotypic material (pop. 172; Fig. 5B). *Desmognathus imitator* is characterized by relatively low levels of mtDNA divergence across its range, a finding in contrast with patterns of high divergence in allozyme profiles over short geographic distances (Tilley 2000). Tilley (2000) discussed a morphologically and genetically distinct series of populations of Imitator Salamanders from higher elevations along the Blue Ridge Parkway in Haywood and Jackson counties, North Carolina. A sample representing these enigmatic populations was included (pop. 181).

In the Bayesian reconstruction, *D. imitator* is sister to all species of *Desmognathus* exclusive of the pygmy salamanders. However, a sister relationship is recovered between *D. imitator* and *D. aeneus* in the ML reconstruction although this relationship is not supported by the bootstrapped ML reconstruction (Fig. 4).

Distribution. This species has a relatively small distribution and, due to our ecoregion X drainage regime, is only represented by four samples. Despite the low level of sampling, the range extent was covered moderately well (Fig. 7).

4. Desmognathus aeneus

A strongly supported clade comprising sixteen populations of *Desmognathus aeneus* was recovered by all phylogenetic reconstruction methods. This clade includes near topotypic material for *D. aeneus* (33 airline km to pop. 135) as well as topotypic material for the synonmized species *D. chermocki* Valentine (pop. 39; Fig. 5C).

In the Bayesian reconstruction, *Desmognathus aeneus* is sister to all species of *Desmognathus* exclusive of the pygmy salamanders and imitator salamanders. As mentioned above, a sister relationship is recovered between *D. imitator* and *D. aeneus* in the ML reconstruction although this relationship is not supported by the bootstrapped ML reconstruction (Fig. 4).

Distribution. Specimens of *Desmognathus aeneus* sequenced herein include many of the same specimens described in Graham *et al.*'s (2012) conservation status paper; as a result the range extent does not differ from that study (Fig.7). The phylogeographic structure within *D. aeneus* is complex and will be addressed in a greatly expanded dataset in another publication.

5-10. Desmognathus quadramaculatus and D. marmoratus

Our phylogenetic reconstructions did not recover a genealogically exclusive lineage of *Desmognathus quadramaculatus* or *D. marmoratus* (Figs. 3, 4). Rather, the 53 populations surveyed show that these two species interdigitate within a strongly supported clade containing all populations of *D. quadramaculatus*, *D. folkertsi* and *D. marmoratus*. Non-monophyly of *D. quadramaculatus* and *D. marmoratus* has been observed in every recent phylogenetic survey that has sampled multiple individuals of both species (Beamer & Lamb 2008; Jones *et al.* 2006; Kozak *et al.* 2005b; Rissler & Taylor 2003; Titus & Larson 1996).

Both Kozak *et al.* (2005b) and Jones *et al.* (2006) reported that within-drainage populations of *Desmognathus quadramaculatus* and *D. marmoratus* are more closely related to each other than to their conspecifics from other drainages. Our phylogenetic reconstructions reveal a different pattern for the more southern populations, in which *D. marmoratus* is genealogically exclusive across multiple drainages and does not nest phylogenetically within co-occurring populations of *D. quadramaculatus*. For example, a *D. marmoratus* sample from population 161 is nested within the *quadramaculatus/marmoratus* C clade while a *D. quadramaculatus* sample collected at the same locality is nested within the *quadramaculatus* A clade (Fig. 8).

The clade comprising *Desmognathus quadramaculatus*, *D. marmoratus* and *D. folkertsi* is characterized by strong phylogeographic structure and is composed of two well-supported subclades. One subclade includes all *D. quadramaculatus* populations sampled south of the Pigeon River as well as all *D. marmoratus* populations sampled in the Apalachicola and Savannah River drainages. The second subclade includes all populations of *D. quadramaculatus* sampled from east of the Tuckasegee River drainage basin (with one exception) as well as all populations of *D. marmoratus* sampled outside the Apalachicola and Savannah River drainage.

5. quadramaculatus A

Kozak et al. (2005b) designated a single population of *Desmognathus quadramaculatus* from Georgia as the clade quadramaculatus/marmoratus A. This clade is represented by fifteen populations of *Desmognathus quadra*-

maculatus in our study, one of which is a topotypic sample (pop.118; Fig. 8) representing the synonmized subspecies *D. q. amphileucus* Bishop.

Distribution. This clade constitutes the southernmost populations of *D. quadramaculatus* (Fig. 9) and is represented by all *D. quadramaculatus* populations sampled south of the Pigeon River, exclusive of those from the Great Smoky Mountains. It demonstrates strong phylogeographic structure in which populations west and southwest of the Little Tennessee River Valley are highly differentiated from those to the south and east.



FIGURE 8. Branch containing *Desmognathus quadramaculatus*, *D. marmoratus* and *D. folkertsi* pruned from Bayesian majority-rule consensus phylogram, diamonds represent posterior probabilities > 0.90. Numbers following species names in parenthesis represent population sample numbers.

6. marmoratus B

Kozak *et al.* (2005b) recovered the *marmoratus* B and *quadramaculatus* A clades as sister taxa. In contrast, our phylogenetic reconstructions recovered a strongly supported sister relationship between *Desmognathus folkertsi* and *marmoratus* B (Figs. 3, 4). Our two samples represent topotypes for two synonmized subspecies, *D. m.aureata*

Martof (pop.106) and *D. m. roborata* Martof (pop. 158; Fig. 8). The close relationship between these synonymized subspecies is corroborated by allozyme data (Voss *et al.* 1995).

Distribution. Representing the southernmost populations of *D. marmoratus*, this clade includes all populations sampled from the Apalachicola and Savannah River drainage basins (Fig.10).



FIGURE 9. Sampling localities for Desmognathus quadramaculatus, symbols match those in Fig. 8.



FIGURE 10. Sampling localities for Desmognathus marmoratus and D. folkertsi, symbols match those in Fig. 8.

7. quadramaculatus/marmoratus C

This strongly supported clade comprises four populations of *Desmognathus quadramaculatus* and five populations of *D. marmoratus*. Four of the populations of *D. marmoratus* are genealogically exclusive despite occupying separate drainage basins and in three cases are sympatric with the *quadramaculatus* A clade (pop. 142, 154, 161; Figs. 8, 9, 10). This stands in stark contrast to the within-drainage pattern of close relationships between *D. quadramaculatus* and *D. marmoratus* reported by Kozak *et al.* (2005b) and Jones *et al.* (2006). Nonetheless, the populations of *D. marmoratus* are nested within other samples of *D. quadramaculatus*. Topotypic specimens representing two synonmized subspecies, *D. m. intermedia* Pope (pop. 191) and *D. m. melania* Martof (pop. 161), are placed within this clade (Fig. 8).

Also included in *quadramaculatus/marmoratus* C is a single population of *D. quadramaculatus* from Carter County, Tennessee (pop. 250) and a single population of *D. marmoratus* from Hickory Nut Gorge, Henderson County, North Carolina (pop. 219). A sister relationship is recovered for these two populations despite being separated by >117 airline km and the main crest of the Blue Ridge Mountains. Together these two samples are sister to the remaining populations of *quadramaculatus/marmoratus* C. Additional sampling in the areas surrounding both of these populations is clearly warranted.

Distribution. Most populations of *Desmognathus quadramaculatus* in *quadramaculatus/marmoratus* C were sampled from the Bald Mountains or adjacent areas of North Carolina (Fig. 9) whereas all population samples of *D*. *marmoratus* were from the Little Tennessee River and Pigeon River drainages (Fig. 10).

8. quadramaculatus D

This well supported clade comprising four populations was recovered by the Bayesian and ML reconstructions (Fig. 8). Kozak *et al.* (2005b) recovered a sister relationship between *quadramaculatus* D and the *quadramaculatus/marmoratus* E clade; we recovered the same relationship herein (Fig. 8).

Distribution. The *quadramaculatus* D clade is known from sites in the New, Roanoke and Pee Dee River drainages (Fig. 9).

9. quadramaculatus/marmoratus E

This strongly supported clade comprises 16 populations of *Desmognathus quadramaculatus* and a single population of *D. marmoratus*. It includes topotypic material for *D. marmoratus* (pop. 259), which is deeply nested within the clade (Fig. 8).

Distribution. The *quadramaculatus/marmoratus* E clade is found in the portions of the Tennessee River drainage north of the French Broad River valley as well as the Santee, New and Pee Dee river drainages (Fig. 9). This clade includes populations of *Desmognathus quadramaculatus* north of the French Broad River valley exclusive of populations in the Bald Mountains.

With the exception of *Desmognathus marmoratus* from the Hickory Nut Gorge (described in the *quadramaculatus/marmoratus* C account), all populations of *D. marmoratus* sampled north of the French Broad River belong to this clade (Jones *et al.* 2006; Kozak *et al.* 2005b). It is worth noting that *D. quadramaculatus* populations that occur at the same site in the Hickory Nut Gorge belong to the *quadramaculatus/marmoratus* E clade.

10. quadramaculatus F

This genealogically exclusive clade is composed of four populations of *D. quadramaculatus* and represents the sister clade to *quadramaculatus* A (Fig. 8). Although *D. quadramaculatus* is well known from the region where these populations were collected (Dodd 2004; Petranka 1998), this clade has not been included in any published phylogenetic reconstructions.

Distribution. *quadramaculatus* F occurs on both sides of the main ridgecrest of the Great Smoky Mountains, although the range extent to the east and west are presently unknown and deserve further study (Fig. 9).

11. Desmognathus folkertsi

Three populations of *Desmognathus folkertsi* were recovered as a genealogical exclusive group (Fig. 8), one of which is within 19.8 airline km of the type locality (pop. 116).

Distribution. These three populations span the known range extent of this narrowly endemic species (Fig. 10; Wooten *et al.* 2010).

12–18. Desmognathus ocoee

The 20 populations we sampled for *Desmognathus ocoee* Nicholls were not genealogically exclusive (Figs. 3, 4); rather, five populations of *D. conanti* are nested within samples otherwise referred to *ocoee* (Fig. 11A). Likewise an additional three populations are part of a clade that also contains *D. apalachicolae* Means and Karlin and *D. conanti* (Fig. 11B). The remaining 12 populations constitute at least two distantly related, topologically disparate clades (Figs. 3, 4). We recovered each of the *D. ocoee* clades identified by Kozak *et al.* (2005b), with the exception of populations referable to their *ocoee* C clade.



FIGURE 11. Branches pruned from Bayesian majority-rule consensus phylogram, diamonds represent posterior probabilities > 0.90. Numbers following species names in parenthesis represent population sample numbers. A. Branch containing some populations of *Desmognathus ocoee*. B. Branch containing *Desmognathus apalachicolae*. C. Branch containing *Desmognathus abditus*. D. Branch containing remaining populations of *Desmognathus ocoee*.

12. ocoee A

This strongly supported clade is sister to a clade containing the *ocoee* B and *ocoee* D clades, a topological profile identical to that recovered by Kozak *et al.* (2005b) (Figs. 3, 4).

Distribution. The ocoee A clade is represented by a single population from the Plott Balsam Mountains (Figs.

11A, 12). Kozak *et al.*'s (2005b) two populations of *ocoee* A were from the Great Smoky Mountains. Both Tilley *et al.* (1978) and Dodd (2004) commented that salamanders herein referred to this clade are generally restricted to high elevations in the Great Smoky Mountains and we believe that it probably occurs at high elevations throughout both the Great Smoky and Plott Balsam mountains. It may contact the *ocoee* B clade in the vicinity of Balsam Gap (Jackson and Haywood counties, North Carolina).

13. ocoee B

Kozak *et al.* (2005b) designated a single population of *Desmognathus ocoee* from Pisgah Ridge in North Carolina as the *ocoee* B clade. This clade is represented by two populations of *Desmognathus ocoee* in our analysis (Fig. 11A), which recovered *ocoee* B as the sister group to the *ocoee* D clade (Figs.3, 4), as noted by Kozak *et al.* (2005b). However, we did not find any samples referable to the *ocoee* C clade, which Kozak *et al.* (2005b) recovered as the closest relative to *ocoee* D.

Distribution. This clade represents the easternmost populations of *Desmognathus ocoee* (Fig. 12). Our two samples were collected from Pisgah Ridge (pop. 189) and the Great Balsam Mountains (pop. 186).



FIGURE 12. Sampling localities for some populations of *Desmognathus ocoee* and *D. abditus*, symbols match those in Fig. 11A, 11C and 11D.

14. ocoee D

ocoee D is a strongly supported clade comprising five populations of *Desmognathus ocoee* and five populations of *D. conanti* (Fig. 11A). This clade includes topotypic material for the junior synonym *D. perlapsus* Neill (pop. 131).

Distribution. Populations of this clade from the southern Appalachians have been considered to represent *Desmognathus ocoee* whereas those from adjacent areas of the Piedmont have been considered to represent *D. conanti* (Jensen 2008). However, it appears that the *ocoee* D clade may be continuously distributed from the headwaters of the Savannah River into the adjacent Apalachicola River drainage and southwards to the vicinity of the fall line in Georgia (Fig. 13). Additional sampling from the Southern Inner Piedmont ecoregion in the upper Apalachicola drainage will be necessary to resolve fine scale distribution and possible clade interactions between *ocoee* D and *D. apalachicolae*.



FIGURE 13. Sampling localities for the some populations of *Desmognathus ocoee* and *D. apalachicolae*, symbols match those in Fig. 11A and Fig. 11B respectively.

One of the populations in Beamer and Lamb (2008) was mapped incorrectly due to a misunderstanding about the collection data. Their population sample 62, reported from Douglas County, Georgia, was actually from Schley County, Georgia, further to the south. Specimens from this locality are nested within *ocoee* D (Beamer and Lamb's locality 62 = pop. 74 herein) and are not assignable to *Desmognathus apalachicolae*.

15. ocoee E

Represented by a single population in Kozak *et al.* (2005b), this strongly supported clade contains seven populations (Fig. 11A). A weakly supported sister relationship between *ocoee* E and a clade comprising the *ocoee* F, G and H clades was recovered in the Bayesian reconstruction (Fig. 3). Conversely, a weakly supported sister relationship between *ocoee* E and a clade containing all populations of *Desmognathus monticola* Dunn was recovered in the bootstrapped ML reconstruction (Fig. 4).

Distribution. This clade occurs in the Cheoah and Nantahala mountains in North Carolina and adjacent Georgia (Fig. 12). Contact between *ocoee* E and the *ocoee* F clade may occur in the Tallulah Creek or Cheoah River valleys in Graham County, North Carolina.

16. ocoee F

Kozak *et al.* (2005) designated a single population of *Desmognathus ocoee* from Graham County, North Carolina, as the *ocoee* F clade. It is represented here by two populations of *Desmognathus ocoee*. All phylogenetic reconstruction methods recovered a strongly supported sister relationship between *ocoee* F and a clade composed of the *ocoee* G and *ocoee* H clades (Figs. 3, 4).

After the phylogenetic analyses presented here were completed, samples representing topotypes for *Desmognathus ocoee* from the Ocoee Gorge were obtained. DNA sequence data for the mtDNA gene *COX1* (~550 bp) was collected and phylogenetic reconstructions of this data recovered the *D. ocoee* topotypes as nested within the *ocoee* F clade. Because this fragment of *COX1* is only ¹/₄ of the size of the mtDNA data fragment we analyzed here, the

topotypic *ocoee* are not shown in the phylogenetic reconstructions presented here. The *COX1* DNA sequence data for the topotypic *D. ocoee* has been accessioned at Genbank. This *COX1* sequence data as well as *COX1* sequence for >1000 additional *Desmognathus* populations will be presented in another series of papers.

Distribution. The *ocoee* F clade is known from the Unicoi (pop. 135) and Snowbird (pop. 142) mountains of North Carolina and the Ocoee River Gorge in Tennessee (Fig. 12).

17. ocoee G

This clade was represented by a single population in Kozak *et al.* (2005b). Our one sample (pop. 75) represents a second population which was collected from the same locality as population 52 in Tilley and Mahoney (1996). It is the sister group to the *ocoee* H clade (Fig. 11D).

Distribution. This clade is only known from two localities, both in the Plateau Escarpment ecoregion of northeastern Alabama (Fig. 12).

18. осоее Н

The *ocoee* H clade was also represented by a single population in Kozak *et al.* (2005b), and is again represented by a single population (pop. 87) in our survey collected from very near population A8 in Anderson and Tilley (2003)(Fig. 11D).

Distribution. This lineage is only known with certainty from two localities in Marion County, Tennessee (Fig. 12). However, Anderson and Tilley's (2003) sampling suggests that it may occur farther north along the Cumberland Plateau.

19. Desmognathus apalachicolae

This strongly supported clade comprises five populations currently referable to *Desmognathus apalachicolae* and includes a sample (pop. 36) from very near the type locality (in the same small stream drainage) (Fig.11B). However, additional populations from northwestern Georgia and adjacent Piedmont currently assigned to *D. ocoee* (n = 3) or *D. conanti* (n = 2) are also included in this clade (Fig. 13). This result is consistent with Beamer and Lamb (2008), who also reported populations of *D. apalachicolae* in the southern Appalachians. The finding that southern Appalachian populations form part of the *D. apalachicolae* clade is not unprecedented; both Valentine (1961) and Means (1974) commented on similarities between Florida panhandle and Georgia mountain populations before *D. apalachicolae* was described (Means & Karlin 1989).

A sister relationship between *Desmognathus apalachicolae* and a clade composed of *D. monticola* and the *ocoee* E, F, G and H clade was recovered (though weakly supported) in both Bayesian and ML reconstructions (Figs. 3, 4).

Distribution. This species was considered to have a small range in the Florida panhandle and southwestern Georgia (Means & Karlin 1989) until Beamer and Lamb (2008) reported populations from the Appalachian Mountains that had previously been identified as *Desmognathus ocoee*. However, it does not appear that the *D. apalachicolae* clade is distributed continuously throughout the Apalachicola River drainage as figured in Beamer and Lamb (2008); instead the middle sections of that drainage are occupied by the *ocoee* D clade (Fig. 13).

20-22. Desmognathus monticola

We recovered a strongly supported, genealogically exclusive clade comprising 83 populations of *Desmognathus monticola* (Fig. 14B). Although Kozak *et al.* (2005b) identified two clades—*monticola* A and *monticola* B—within this widespread species, our phylogenetic reconstruction recovered an additional clade that is sister to all remaining *monticola* populations (Fig. 14B). In general, our results mirror those of Bonett *et al.* (2007), who reported that most of the genetic diversity in *D. monticola* is restricted to the southern portions of its range.

20. monticola A

Kozak *et al.* (2005b) designated a single sample from Butler County, Alabama, as *monticola* A. It is represented by 11 populations herein (Fig. 14B). A sister relationship was recovered between the *monticola* A and *monticola* B clades by all phylogenetic reconstruction methods (Figs. 3, 4.).

Distribution. Populations of *monticola* A occur within two widely separated geographic areas, one in southern Alabama and the second in northern Georgia and Polk County, Tennessee (Fig. 15).



FIGURE 14. Branches pruned from Bayesian majority-rule consensus phylogram, diamonds represent posterior probabilities > 0.90. Numbers following species names in parenthesis represent population sample numbers. A. Branch containing all populations of *Desmognathus carolinensis* and some populations of *D. fuscus*. B. Branch containing *Desmognathus monticola*.

21. monticola B

Other studies (Bonett *et al.* 2007; Kozak *et al.* 2005b; Rissler & Taylor 2003) have found that populations of *Desmognathus monticola* occurring in the northern portion of its range, from North Carolina to Pennsylvania, are characterized by extreme genetic homogeneity. Our phylogenetic reconstruction also reveals limited genetic differentiation in *monticola* B (Fig. 14B) and includes topotypic material for the species (pop. 194) as well as the synonmized subspecies *D. m. jeffersoni* (pop. 360).

Distribution. The northern range extent of this clade appears to coincide with that presented for *D. monticola* in Petranka (1998). The southern range extent may be in the vicinity of the Hiwassee River in northern Georgia and adjacent North Carolina and Tennessee (Fig. 16).



FIGURE 15. Sampling localities for southern populations of Desmognathus monticola, symbols match those in Fig. 14B.



FIGURE 16. Sampling localities for northern populations of Desmognathus monticola, symbols match those in Fig. 14B.

22. monticola C

This well supported clade contains three populations and is sister to a clade composed of the *monticola* A and B clades (Fig. 14B). It has not been reported in any other published molecular phylogenetic reconstructions.

Distribution. This clade appears to be restricted to a small area in the Talladega Upland ecoregion of Alabama (Fig. 15), which continues into western Georgia. Thus, it is possible that *monticola* C occurs in adjacent western Georgia as well, though no specimens representing this clade were collected from that state.

23. Desmognathus carolinensis

A genealogically exclusive *Desmognathus carolinensis* Dunn was not recovered in either phylogenetic analysis. The ten populations of *D. carolinensis* sampled in the survey formed a clade with 39 populations of *D. fuscus* (Fig. 14A). A close relationship among haplotypes between certain populations of *D. fuscus* and *D. carolinensis* has been reported several times (Beamer & Lamb 2008; Kozak *et al.* 2005b; Tilley *et al.* 2008, 2013). Populations of the *Desmognathus carolinensis* clade exhibit moderate levels of mtDNA divergence; the clade is represented by topotypic material (pop. 227). Tilley *et al.* (2013) has referred to some populations of *D. fuscus* with *D. carolinensis* mtDNA as the "Sinking Creek Form" including populations from very near our population 250.

Distribution. This species has a relatively small range in the southern Blue Ridge Mountains and our ten samples extend across much of the range extent (Fig. 17). However, the populations of *Desmognathus fuscus* containing *carolinensis* mtDNA are much more widely distributed than previously known (Fig. 17); their geographic distribution and puzzling genetic constitution will be discussed in another manuscript.



FIGURE 17. Sampling localities for all populations of *Desmognathus carolinensis* and populations of *D. "fuscus"* with *D. carolinensis* mtDNA haplotypes, symbols match those in Fig. 14A.

24. Desmognathus abditus

Three populations of *Desmognathus abditus* Anderson and Tilley were recovered as a genealogical exclusive group in all of our phylogenetic reconstructions (Fig. 11C); one of these populations (pop. 124) represents topotypic material. The topological position of *D. abditus* relative to other species is not well resolved by either the Bayesian or the ML phylogenetic reconstructions (Figs. 3, 4).

Distribution. This species has a small range and appears to be restricted to southern portions of the Cumberland Plateau (Anderson & Tilley 2003) (Fig. 12).

25. Desmognathus brimleyorum

A strongly supported genealogically exclusive clade comprising four populations of *Desmognathus brimleyorum* was recovered by all phylogenetic reconstruction methods (Fig. 18D). A sister relationship between *Desmognathus brimleyorum* and a clade comprising the *conanti* A, B, C, D and E clades, *D. santeetlah* Tilley, and the valentinei Means, Lamb and Bernardo clade is recovered by both the Bayesian and ML reconstructions (Figs. 3, 4). However, support for this relationship is weak.

Distribution. This species is primarily known from the Ouachita Uplift in Arkansas and Oklahoma (Trauth *et al.* 2004), although Kozak *et al.* (2005b) reported it from the coastal plain of Arkansas. We also sampled a population referable to this species from the coastal plain of Arkansas (pop. 15; Fig. 19), which suggests that *D. brimleyorum* might also occur in adjacent portions of northern Louisiana. These findings call into question whether *Desmognathus conanti* occurs in southern Arkansas.



FIGURE 18. Branches pruned from Bayesian majority-rule consensus phylogram, diamonds represent posterior probabilities > 0.90. Numbers following species names in parenthesis represent population sample numbers. A. Branch containing all populations of *Desmognathus ochrophaeus* and *D. orestes*. B. Branch containing northern populations of *Desmognathus auriculatus*. C. Branch containing *Desmognathus valentinei*. D. Branch containing *Desmognathus brimleyorum*. E. Branch containing *Desmognathus welteri*. F. Branch containing southern populations of *Desmognathus auriculatus*. G. Branch containing *Desmognathus planiceps*.



FIGURE 19. Sampling localities for *Desmognathus brimleyorum*, *D. valentinei* and populations of *D. conanti* west of the Mississippi River, symbols match those in Fig. 18D, Fig. 18C and Fig. 20A respectively.

26-31. Desmognathus conanti and D. santeetlah

We did not recover a genealogically exclusive *Desmognathus conanti*; instead, a clade comprising ten populations of *D. santeetlah* is nested within our 88 population samples of *D. conanti* (Figs. 3, 4). A close relationship between *D. conanti* and *D. santeetlah* has been noted in other molecular phylogenetic surveys (Beamer & Lamb 2008; Kozak *et al.* 2005b; Tilley *et al.* 2013; Tilley & Schwerdtfeger 1981; Titus & Larson 1996).

26. conanti A

Kozak *et al.* (2005b) designated two populations of *Desmognathus conanti* from South Carolina as the clade *conanti* A. This clade is represented by 29 populations of *D. conanti* in our survey (Fig. 20B). A sister relationship between the *conanti* A clade and a clade composed of *Desmognathus santeetlah* and all other populations of *D. conanti* was recovered by all phylogenetic methods (Figs. 3, 4).

Tilley *et al.* (2013) designated two clades of salamanders, the β clade and γ clade and recovered a sister relation to clades of *conanti*-like salamanders. They concluded that the β clade and γ clade represent "failed" species, (i.e., they exchange genes with other clades at levels that compromise their evolutionary independence). Tilley *et al.* (2013) sequenced a different mitochondrial gene (*CYT-B*), which precludes including their sequence data in the data matrix analyzed here. Therefore it is not known if any samples from their β clade and γ clade are present in our phylogenetic reconstruction. However, we note that our *conanti* A clade is also sister to a clade of *conanti*-like salamanders, a similar topological position to the β clade and γ clade in the phylogenetic reconstruction presented by Tilley *et al.* (2013).

Distribution. All populations of *Desmognathus conanti* in the Atlantic drainages of South Carolina and Georgia, as well as those from the headwaters of the French Broad River in North Carolina, belong to the *conanti* A clade (Fig. 21). Exhibiting considerable genetic diversity and phylogeographic structuring, this clade warrants further investigation.



FIGURE 20. Branches pruned from Bayesian majority-rule consensus phylogram, diamonds represent posterior probabilities > 0.90. Numbers following species names in parenthesis represent population sample numbers. A. Branch containing most Gulf drainage populations of *Desmognathus conanti*. B. Branch containing all Atlantic drainage populations of *Desmognathus conanti* as well as populations in the headwaters of the French Broad River drainage. C. Branch containing *Desmognathus santeetlah*.

27. conanti B

conanti B is a strongly supported genealogically exclusive clade comprising five populations recovered by all phylogenetic reconstruction methods (Fig. 20A). Kozak *et al.* (2005b) recovered a sister relationship between *conanti* B and a clade comprising the *conanti* C and D clades. We recovered this same relationship in Bayesian phylogenetic reconstructions (Fig. 3) but our bootstrapped ML reconstruction did not resolve relationships between the *conanti* B, C + D and E clades (Fig. 4).

Distribution. Populations sampled from the Florida parishes of Louisiana and adjacent areas in Mississippi in the Tangipahoa, Amite, Bayou Sara, and lower Homochitto river drainages belong to the *conanti* B clade (Fig. 21).



FIGURE 21. Sampling localities for *Desmognathus santeetlah* and all populations of *D. conanti* from east of the Mississippi River, symbols match those in Fig. 20A, Fig. 20B and Fig. 20C.

28. conanti C

This clade was represented by a single population in Kozak *et al.* (2005b), who also noted that it was closely related to another clade they designated as *auriculatus*. However, Beamer and Lamb (2008) demonstrated that Kozak *et al.*'s (2005b) *auriculatus* samples from the Gulf coastal plain were not referable to *Desmognathus auriculatus*. Here we recognize a single clade for those samples representing Kozak *et al.*'s (2005b) *conanti* C and *auriculatus* clades; strongly supported, and genealogically exclusive, it comprises 14 populations (Fig. 20A). The sister relationship between the *conanti* C (+ *auriculatus*) and *conanti* D clades recovered by Kozak *et al.* (2005b) was recovered in our Bayesian phylogenetic reconstructions (Fig. 3).

Distribution. This clade includes all populations of *Desmognathus conanti* sampled from the following river drainages in the coastal plain of Mississippi, Alabama and Florida: lower Choctawhatchee, Yellow, Escambia, lower Mobile, Pascagoula and Pearl (Fig. 21).

29. conanti D

Kozak *et al.* (2005b) designated 11 populations of *Desmognathus conanti* as the clade *conanti* D, represented herein by 22 populations. Specimens collected from the type locality of *D. conanti* (pop. 70) are nested within this clade (Fig. 20A).

Distribution. Our populations of *Desmognathus conanti* from the Tennessee River drainage (excluding those aforementioned in association with the *conanti* A, as well as populations from the upper Mobile and Yazoo river drainages, belong to *conanti* D (Fig. 21).

30. conanti E

A genealogically exclusive clade comprising five populations (Fig. 20A) of *D. conanti* was recovered as the sister group to a clade including the *conanti* B, C and D clades (Fig. 3). Although this clade was detected previously by Beamer and Lamb (2008), this is the first time that *conanti* E has been placed in a comprehensive phylogenetic context.

We sought but were unable to obtain samples of *Desmognathus* from eastern Texas, which represents the western geographic terminus for the genus. Following completion of our fieldwork, Hibbitts *et al.* (2015) located four populations of *Desmognathus* within its historical Texas range; their Bayesian analysis of *COX1* sequence data corroborates our findings that *Desmognathus* from the western extent of the Coastal Plain are not *D. auriculatus* but rather *D. conanti*. Hibbitts *et al.*'s (2015) molecular phylogenetic survey incorporated GenBank sequences from Beamer and Lamb's (2008) Louisiana localities (i.e., the populations that constitute this clade). Their Texas samples form a clade with these Louisiana populations, and thus we assign their four Texas populations to *conanti* E.

Distribution. All of our samples of *Desmognathus conanti* from west of the Mississippi River belong to this clade (Fig. 19). Four of the populations are from the Red River drainage while the last population is from the Bayou Teche drainage. As mentioned above, all known extant *Desmognathus* populations from Texas appear to represent this clade.

31. Desmognathus santeetlah

We recovered a strongly supported clade comprising 11 populations of *Desmognathus santeetlah* (Fig. 20C), which includes a sample representing near topotypic material (pop. 128). *Desmognathus santeetlah* is the sister clade to all populations of *D. conanti* exclusive of *conanti* A (Figs. 3, 4).

Distribution. The distribution of *Desmognathus santeetlah* as sampled herein does not differ substantially from that of the outlined species description (Tilley 1981; Fig. 21). Low elevation populations from both sides of the Great Smoky Mountains' main ridgecrest have been referred to *D. conanti* (Dodd 2004), but populations from the same locations in our survey possessed haplotypes that nested within the *D. santeetlah* lineage. This result is not a surprising because the two species commonly hybridize in this area, although the species status of "*conanti*" in this area is unresolved (Tilley 1988; Tilley *et al.* 2013).

32–34. Desmognathus auriculatus

We sampled 33 populations currently referred to *Desmognathus auriculatus* (Beamer & Lamb 2008; Conant & Collins 1998; Means *et al.* 2017; Petranka 1998). These populations were not recovered as a genealogically exclusive group. Populations currently referred to *D. auriculatus* occupy at least two topologically disparate positions in our phylogenetic reconstructions (Figs. 3, 4), confirming the polyphyletic profile we had detected previously (Beamer & Lamb 2008).

32. auriculatus A

A well-supported clade, *auriculatus* A, is composed of four populations and was recovered in both Bayesian and ML reconstructions (Fig. 18F). A sister relationship between *auriculatus* A and a clade containing two additional clades of *auriculatus*, *D. fuscus* Rafinesque, and *Desmognathus planiceps* Newman was recovered by the Bayesian phylogenetic reconstruction (Fig. 3). However, the ML reconstruction identified a sister relationship strictly between *auriculatus* A and *D. planiceps*, though it is not supported in the bootstrapped analysis (Fig. 4).

This clade includes a sample from the vicinity of the type locality of *D. auriculatus* (pop. 84; Fig. 22). Repeated recent searches at the type locality in the vicinity of Riceboro, Georgia suggest that this species may no longer be extant in that area (Graham *et al.* 2010). The decline of this species makes the resolution of the proper assignment for *auriculatus* problematic. An apparent break between the *auriculatus* A and *auriculatus* B clades occurs at the Ogeechee River. The type locality occurs south of the Ogeechee River and all samples collected south of the Ogeechee River are recovered in the *auriculatus* A clade.

Distribution. This clade occurs from south of the Ogeechee River in Georgia, to at least the Suwanee River drainage in Florida (Fig. 22). The western range extent of this clade is unknown.

33. auriculatus B

This strongly supported clade is composed of 12 populations (Fig 18B), Beamer and Lamb (2008) included two samples (their localities 15, 16) of this clade in their phylogenetic reconstruction. A sister relationship between the *auriculatus* B and C clades is recovered by all phylogenetic reconstruction methods (Figs. 3, 4).

Distribution. This clade occupies portions of the coastal plain in South Carolina south of the Pee Dee River to the eastern banks of the Ogeechee River in Georgia (Fig. 40).

34. auriculatus C

This clade contains the northernmost samples currently referred to *D. auriculatus* and is composed of 15 populations (Fig. 18B), two of which are widely disjunct, occurring outside the Coastal Plain in the Blue Ridge Mountains of Virginia (pop. 294 and 295). Tilley *et al.* (2008) were the first to report this montane population (their populations 18 & 19). However, they did not have the phylogenetic context to identify its close genetic relationship to populations from North Carolina's coastal plain. The Blue Ridge salamanders bear no morphological resemblance to coastal plain populations and occupy a grossly different habitat.

The Blue Ridge haplotypes are sister to all other *auriculatus* C haplotypes, and the gap between their disjunct distributions is inhabited by other clades in the *Desmognathus fuscus* complex. It is unlikely that the Blue Ridge populations were introduced given their morphological distinctiveness and level of haplotype divergence. Instead we hypothesize that either these populations represent the ancestral founders of the coastal plain populations or that this pattern represents the historical remnants of ancient hybridization at a time when the *auriculatus* C clade might have been distributed much farther inland than at present.

Distribution. In general, the *auriculatus* C clade is found north of the Great Pee Dee River, but we have found a single population immediately south of the Great Pee Dee River (pop. 271; Fig. 22).



FIGURE 22. Sampling localities for Desmognathus auriculatus, symbols match those in Fig. 18A and Fig. 18F.

35. Desmognathus valentinei

Desmognathus valentinei is represented by four populations that are recovered as the sister group to a clade comprised of all *conanti* and *santeetlah* samples by each of the phylogenetic reconstruction methods (Figs. 3, 4). The easternmost population (pop. 21) is highly differentiated from the others (Fig. 18C).

Distribution. This species is represented by four populations collected west of Mobile Bay (Fig. 19) and represents, at least in part, salamanders that were described as "pale *auriculatus*" by Valentine (1963). There is a large sampling hiatus between our easternmost *valentinei* sample (west of Mobile Bay) and samples of *Desmognathus auriculatus* in Florida. The phylogenetic status of intervening populations of lowland swamp dwelling dusky salamanders is unknown; unfortunately, they have experienced severe declines and may be extirpated (Means & Travis 2007).

Our population 21 is the easternmost known locality for *D. valentinei*. A photograph of a specimen found at this site was presented in Means *et al.* (2017) as their figure 11c. However the authors did not have specimens or sequence data from this site and they use ambiguous language stating "In addition, the species may still range further east into Baldwin and Mobile counties, Alabama." The sample representing our population 21 is sister to all other populations of *D. valentinei* although it is highly differentiated genetically. We assign this sample to *D. valentinei* but clearly additional investigation into populations from this general area is warranted.

36–39. Desmognathus fuscus

Our 88 population samples of *Desmognathus fuscus* represent a paraphyletic group within an otherwise wellsupported clade containing two additional lineages, *auriculatus* B and *auriculatus* C (Figs. 3, 4); paraphyly of *D*. *fuscus* was noted previously by Beamer and Lamb (2008).



FIGURE 23. Branches pruned from Bayesian majority-rule consensus phylogram, diamonds represent posterior probabilities > 0.90. Numbers following species names in parenthesis represent population sample numbers. A. Branch containing some populations of *Desmognathus fuscus*. B. Branch containing additional populations of *Desmognathus fuscus*. C. Branch containing remaining populations of *Desmognathus fuscus*.

36. fuscus A

Kozak *et al.* (2005b) designated two populations of *Desmognathus fuscus* from Tennessee as *fuscus* A. This clade, represented by ten populations of *Desmognathus fuscus* in our study (Fig. 23C), is the sister group to a clade comprising the *fuscus* B, *fuscus* D, *auriculatus* B, and *auriculatus* C clades (Figs. 3, 4).

Distribution. The geographic range of *fuscus* A is considerably larger than previously known, extending as far west as Trigg County, Kentucky (pop. 71) and as far east as Scott County, Virginia (pop. 244; Fig. 24). Population sampling for this clade was sparse given the concomitantly low number of level IV ecoregions across the presumed range. However, its potential for contact zones with the *fuscus* B clade deserves further attention.



FIGURE 24. Sampling localities for *Desmognathus fuscus* (exclusive of those populations characterized by *D. carolinensis* mtDNA haplotypes), symbols match those in Fig. 23.

37. fuscus B

Kozak *et al.* (2005b) designated seventeen populations of *Desmognathus fuscus* as the *fuscus* B clade. A sister relationship between this strongly supported clade (43 populations) and the *fuscus* D clade is recovered in all phylogenetic reconstructions (Figs. 3,4). A precise type locality was not given in the original description of *D. fuscus*, being stated simply as "in the northern parts of the state of New York, in small brooks" (Rafinesque 1820). Our sample (pop. 378) collected from this general area is nested deeply within the clade (Fig 23A).

Distribution. The *fuscus* B clade is the most widespread clade in the entire genus, ranging from near the North Carolina-Virginia border northwards into Canada (Fig. 24). Tilley *et al.* (2013) identified the southeastern range limits of this clade, which is identified as "*Desmognathus fuscus* clade" in the terminology used in their publication. Across this range, populations occur from high elevation montane habitats to coastal plain wetlands. We have only a few samples from the more northern sections of the range because populations there are characterized by low genetic diversity (Karlin & Guttman 1986).

38. fuscus C

All phylogenetic reconstructions recovered a sister relationship between *fuscus* C and a clade containing the *fuscus* lineages A, B, and D as well as lineages *auriculatus* B and C (Figs. 3, 4). The *fuscus* C lineage contains at least two well supported clades (Fig. 23B) and should receive further attention towards understanding its interactions with other lineages in the *fuscus* complex as well as with *conanti* A, which occurs in nearby areas of western South Carolina. Certain populations of *fuscus* C are characterized by very large, robust salamanders resembling the populations of *Desmognathus fuscus* that possess *carolinensis* mtDNA (See account 23).

Distribution. Populations of this clade were collected primarily in South Carolina, although we collected a single population in North Carolina (pop. 255). The *fuscus* C clade occurs from the Piedmont east into the adjacent Coastal Plain (Fig. 24).

39. fuscus D

We sampled 15 populations of this clade, which is highly differentiated from *fuscus* B (Fig. 23A). The *fuscus* D clade was recognized by Kozak *et al.* (2005b) in their maximum lineage partitioning analyses, but they apparently confused a sample from the coastal plain of North Carolina with another from Massachusetts. This mistake made it appear that coastal plain populations from North Carolina (which they considered to represent *Desmognathus auriculatus*) were only slightly differentiated from those in the northeastern United States.

Distribution. This lineage occurs in the Piedmont and upper Coastal Plain of North Carolina, adjacent areas of Virginia, and has been located at a single site in South Carolina. There is an apparently disjunct population in the Blue Ridge Mountains of Virginia (pop. 354; Fig. 24).

40. Desmognathus planiceps

The placement of *Desmognathus planiceps* differs slightly across our phylogenetic reconstructions. In the Bayesian reconstruction, *D. planiceps* is the sister group to a clade containing *auriculatus* B and *auriculatus* C as well as all the *fuscus* lineages (Fig. 3). Conversely, the ML analysis depicts a sister relationship between *D. planiceps* and *auriculatus* A whereas the bootstrapped ML reconstruction recovers a polytomy for all of these lineages (Fig. 4). These results differ from Tilley *et al.*'s (2008) phylogeny which recovered a sister relationship between *planiceps* and some populations of *fuscus*, however their analysis did not include samples of *D. auriculatus*. The three population samples of *D. planiceps* are genealogically exclusive and include topotypic material (pop. 304; Fig. 18G).

Distribution. The range of our samples does not differ significantly from that outlined in Tilley *et al.* (2008), although one of our three populations (pop. 321) extends the range further north than has previously been documented (Fig. 25). The *planiceps* clade provides one of the few examples in this study (outside of populations of *D. monticola*) where a haplotype is shared between two localities—in this case, populations 304 (the topotypic sample) and 379 (~8.5 airline km away).

41. Desmognathus welteri

A sister relationship between *Desmognathus welteri* Barbour and a clade containing *D. planiceps*, all *fuscus* clades, and the *auriculatus* A, B, and C clades is recovered in all phylogenetic reconstructions (Figs. 3, 4.) The four population samples of *D. welteri* are genealogically exclusive and a topotypic sample (pop. 249) is nested within this clade (Fig. 18E). Originally described as a subspecies of *D. fuscus* (Barbour 1950), *D. welteri* was recognized as a distinct species following a detailed comparative analysis of larval morphology (Rubenstein 1971). Its specific status is strongly supported by molecular data (Beamer & Lamb 2008; Kozak *et al.* 2005b; Rissler & Taylor 2003; Titus & Larson 1996).

Distribution. The distribution of *Desmognathus welteri* as sampled in this study (Fig. 25) does not differ from the previously described range.

42-45. Desmognathus orestes and D. ochrophaeus

A paraphyletic *Desmognathus orestes* Tilley and Mahoney was recovered in all of our phylogenetic reconstructions (Figs. 3, 4, 18A). In the species description of *D. orestes*, Tilley and Mahoney (1996) noted the existence of two differentiated clades, designated *orestes* B and C. Salamander populations referable to *orestes* B and C have since been the subject of two phylogenetic surveys (Kozak *et al.* 2005b; Mead *et al.* 2001), generating discrepancy with regard to clade nomenclature. Both Tilley and Mahoney (1996) and Mead *et al.* (2001) referred to these two clades as *orestes* B and C; Kozak *et al.* (2005b) subsequently assigned these clades the names *orestes* A and B. The clade designation *orestes* B refers to the same assemblage in all three publications; hence we follow this designation. Since Tilley and Mahoney's (1996) *orestes* C clade has been used more extensively in the literature and proceeds Kozak *et al.*'s (2005b) *orestes* A, we use the name *orestes* C in reference to those populations occupying the more northern reaches of the species' range extent.



FIGURE 25. Sampling localities for *Desmognathus welteri* and *D. planiceps*, symbols match those in Fig. 18E and Fig. 18G respectively.

42. orestes A

We use the name *orestes* A to refer to a clade comprising four populations that have yet to be subject to any molecular phylogenetic survey. However, they are here considered to represent *D. orestes* given their proximity to other populations that have been characterized genetically (Tilley & Mahoney 1996). In each of the phylogenetic reconstructions, *orestes* A is sister to a clade containing *D. ochrophaeus* Cope and the *orestes* B and *orestes* C clades (Figs. 3, 4, 18A).

Distribution. This clade occurs further south and east than other clades of *D. orestes* and appears to be restricted to slopes draining towards the Atlantic (Fig. 26). Populations are known from the Atlantic draining slopes of Grandfather Mountain, the South Mountains and along the Blue Ridge Escarpment in portions of the Pee Dee River headwaters

43. orestes B

This strongly supported clade comprising four populations was recovered as the sister clade to *orestes* C in all phylogenetic reconstructions (Figs. 3, 4, 18A).

Distribution. Haplotypes composing the *orestes* B clade were only sampled in the Nolichucky and Watauga River drainages of North Carolina and Tennessee (Fig. 26). It is important to reiterate the discrepancy between some populations assigned to *orestes* B (Tilley & Mahoney 1996) and the clade recognized in this study. Samples of *orestes* B reported by Tilley and Mahoney (1996) were found either along the North Carolina-Tennessee border or further west in Tennessee. We recovered mtDNA haplotypes nested within *orestes* B in areas that Tilley and Mahoney (1996) assigned to *orestes* C on the basis of allozymes which might represent historical introgression between these clades.



FIGURE 26. Sampling localities for Desmognathus ochrophaeus and D. orestes, symbols match those in Fig. 18A.

44. orestes C

Populations from the type locality of *Desmognathus orestes* (pop. 282) are assignable to the *orestes* C clade (Fig. 18A).

Distribution. Populations with *orestes* C haplotypes are largely congruent with populations assigned to *orestes* C in Tilley and Mahoney (1996). However, they found populations referable to *orestes* C in the Nolichucky, Watauga, and Santee River drainages; our samples from those areas belong to other mtDNA clades (Fig. 26).

45. ochrophaeus

A genealogically exclusive clade comprising 11 populations of D. ochrophaeus was recovered by all phylogenetic reconstruction methods (Fig. 18A). This clade includes a population from Potter County, Pennsylvania, some190 km west of the type locality in Susquehanna County. Although that is a considerable distance from the type locality, populations of D. ochrophaeus are characterized by genetic homogeneity in the northern portions of their range (Tilley & Mahoney 1996). This 'near' topotypic sample was nested within this clade.

Distribution. *Desmognathus ochrophaeus* was sampled sparsely because large portions of its northern range are characterized by genetic homogeneity (Tilley & Mahoney 1996). Nonetheless, most of the range extent was sampled (Fig. 26).

Strengths of the dataset. The major focus of our survey was to deploy a sampling regime that—used in conjunction with appropriate molecular markers—should identify and delimit all evolutionary independent clades in *Desmognathus*. Previous documentation of low vagility and phylogeographic structure in these and other plethod-ontid salamanders (García-París *et al.* 2000; Jockusch & Wake 2002; Tilley & Mahoney 1996) strongly influenced our design of a sampling grid defined by level IV ecoregions and independent river drainages. We hypothesized that genetic breaks among clades should be coincident with certain of the ecoregion X drainage units. Not knowing which sample units might serve as barriers to gene flow provided the rationale to collect representative material from each and every sample unit.

We argue that our ecoregion X drainage grid performed well in clade identification and delimitation. Our reasoning is two-fold. First, we corroborated results of Kozak *et al.* (2005b), effectively recovering their same combination of 34 described species/undescribed clades. Second, by building on Kozak *et al.*'s (2005) tree-based approach, we identified an additional 12 clades, raising the number of independent evolutionary clades to 45 (an increase of 27%). Actually, the number of recovered clades is proportionately small relative to the number of populations sampled. Thus, we argue that the geographic design of our sampling effort as opposed to a mere increase in sample site number was the key to a more thorough clade recovery.

It bears mention that our ecoregion X drainage grid did not detect some recently identified clades including the β clade and γ clades described in Tilley *et al.* (2013). Our focus was to collect in each level IV ecoregion in each independent drainage. The ecoregions and drainages where the β clade and γ clade occur were sampled; however, in each case that same ecoregion X drainage unit is large and subdivided by other ecoregions. An effort was not made to sample each separate subdivided ecoregion although that approach may clearly be warranted.

A noteworthy benefit of ecoregion X drainage sampling for phylogenetic reconstruction is that this approach lends itself to generating and testing phylogeographic hypotheses. For example, most clades of *Desmognathus* are distributed parapatrically to respective sister groups or closely related clades. The biologically realistic boundaries between ecoregion X drainage units may represent potential barriers to gene flow, whether within or between clades. Testing phylogeographic hypotheses generated from such a dataset stands to help distinguish the roles of contemporary versus paleogeographic boundaries with regard to distribution and diversity.

Sampling level IV ecoregions may provide additional benefits: they were developed as a spatial framework for environmental resource management. Since the more pressing environmental needs include developing regional biological criteria, water resource standards, and nonpoint-source pollution management, ecoregion X drainage sampling could help provide important conservation measures for salamanders and other co-distributed taxa in the eastern U.S.

We suggest that ecoregion X drainage sampling would be appropriate for systematic inventory of several eastern U.S. taxa similarly characterized by low vagility and large range extents. Exemplars include other plethodontid taxa as well as a wide array of terrestrial gastropods and arthropods (spiders, millipedes, insects, etc.). For example, implementation of ecoregion X drainage sampling may provide additional resolution and reveal additional clades beyond those recovered in landmark phylogenetic surveys of eastern U. S. trapdoor spiders (Hendrixson & Bond 2005) and millipedes (Marek 2010; Walker *et al.* 2009).

Limitations of mtDNA phylogenetic reconstructions and species inference. Although mtDNA has been used extensively in phylogenetic surveys, its application in species delimitation has been repeatedly challenged (Hamilton *et al.* 2014; Hebert & Gregory 2005; Sites & Crandall 1997; Wiens & Penkrot 2002). Much of this debate involves issues related to gene tree heterogeneity, which can result from incomplete lineage sorting, introgression, gene duplication, and even differences in gender-mediated gene flow (Edwards 2009; Maddison 1997). Gene tree heterogeneity is common in salamanders, a taxon for which mtDNA frequently over-resolves species boundaries (Highton 2014a; Jockusch *et al.* 2012; Jockusch & Wake 2002; Martínez-Solano *et al.* 2007, 2012). Jockusch & Wake (2002) hypothesized that certain features of salamander biology can contribute to mitochondrial versus nuclear gene discordance, as follows: 1) low vagility is conducive to genetic fragmentation; 2) greater female philopatry results in male-mediated gene flow and, thus, more rapid mixture of nuclear genes; and 3) slow divergence in mate recognition systems facilitates hybridization upon secondary contact. Such patterns have been reported for both slender salamanders (*Batrachoseps*—(Martínez-Solano *et al.* 2007, 2012) and woodland salamanders (genus *Plethodon*—(Highton 1989, 2014b; Highton & Peabody 2000; Weisrock *et al.* 2005; Weisrock & Larson 2006; Wiens *et al.* 2006).

Unlike *Batrachoseps* and *Plethodon*, a comprehensive, range-wide survey of nuclear gene sequences has yet to be conducted for *Desmognathus*. Although a considerable allozymic database exist (Anderson & Tilley 2003; Bonett 2002; Camp *et al.* 2002; Karlin *et al.* 1993; Karlin & Guttman 1981, 1986; Means & Karlin 1989; Tilley 2000, 2016; Tilley *et al.* 1978, 2013; Tilley & Mahoney 1996; Voss *et al.* 1995), none of these studies included samples of all or even most species. However, the few studies of *Desmognathus* that have combined mtDNA and allozymic data to evaluate species boundaries (Mead *et al.* 2001; Tilley *et al.* 2008, 2013) have revealed patterns similar to those for *Batrachoseps*.

An important commonality of the many allozyme studies on *Desmognathus* is their detection of gene flow between species (Anderson & Tilley 2003; Bonett 2002; Karlin & Guttman 1981; Mead *et al.* 2001; Tilley 1988,

2016; Tilley *et al.* 2013; Tilley & Mahoney 1996; Voss *et al.* 1995). Of the 21 currently recognized species, ten have been documented to hybridize with one or more congeners. For example, three species, minimally, hybridize with *D. carolinensis* (Beamer & Lamb 2008; Kozak *et al.* 2005b; Mead *et al.* 2001; Tilley *et al.* 2013; Tilley & Mahoney 1996). Table 2 highlights documented cases of reticulation in *Desmognathus* in terms of the mtDNA clades we recovered, and it is apparent that reticulation often involves distantly related mtDNA clades. Tilley (2016) presents additional instances of reticulation involving clades that can't be directly linked to those sampled in the present study (i.e. the exact populations were not sampled in both studies).

TABLE 2.	Cases of hybridiz	ation identifie	d in l	iterature	that can	be	confidently	assigned to	o the	mtDNA	clades	in the
present stu	dy.											

Lineage 1	Lineage 2	publication	
ochrophaeus	fuscus B	Karlin & Guttman 1981	
fuscus A	conanti D	Bonett 2002	
ochrophaeus	abditus	Anderson & Tilley 2003	
ocoee FGH	abditus	Anderson & Tilley 2003	
carolinensis	orestes B	Mead & Tilley 2000	
orestes B	orestes C	Mead et al. 2001	

Given expectations of gene tree heterogeneity and widespread phylogenetic reticulation in *Desmognathus*, we do not consider it prudent to make species-level inferences from this mitochondrial DNA dataset. Although the evolutionary rates that characterize vertebrate mitochondrial genomes are suitable for reconstruction of the evolutionary history of *Desmognathus* (Kozak *et al.* 2009; Vieites *et al.* 2007; Wiens 2007), using our data for species delimitation is further compromised by the fact that only a single individual was sampled per population. This singular sampling makes it impossible to detect sympatry between clades—a hallmark of speciation.

Taxonomic comments. Despite the dataset's taxonomic limitations we do offer some minor amendments, namely, redefining certain traditionally-recognized species complexes to reflect clade relationships revealed by our phylogenetic analyses. However, even these taxonomic changes should be considered works in progress and may be subject to further (ongoing) revision. The order of species/clade groups presented in this section corresponds to clade presentation in the Results section.

Desmognathus imitator

Although *Desmognathus imitator* is sometimes considered to be a member of the *D. ochrophaeus* complex (Petranka 1998), it is only distantly related to the clade containing *D. ochrophaeus*. Thus, it should no longer be considered a member of a closely related, morphologically homogeneous group that includes *D. ochrophaeus* (or other former members of this complex).

Desmognathus aeneus

A population of *Desmognathus aeneus* near the southern extent of the range in Alabama was originally described as a separate species, *Desmognathus chermocki* (Bishop & Valentine 1950). Although more than one species may be represented among populations currently considered to be *D. aeneus*, the more genetically divergent populations do not occur at the southern extent of the range. As such, it would be premature to assign to any of the 16 populations surveyed herein to *D. chermocki*.

Desmognathus quadramaculatus complex

The evolutionary histories of *Desmognathus quadramaculatus*, *D. marmoratus* and *D. folkertsi* are closely intertwined, and because the specific status of many populations of *D. quadramaculatus* and *D. marmoratus* will require further investigation, the seven clades are best addressed as a species complex.

quadramaculatus A

Although Kozak *et al.* (2005b) referred to this clade as *quadramaculatus/marmoratus* A, it appears to consist solely of salamanders matching the morphological description of *Desmognathus quadramaculatus*. The clade *quadramaculatus* A is sympatric with two additional clades within the complex that match the morphological de-

scription of *D. marmoratus* (*marmoratus* B and *quadramaculatus/marmoratus* C) although they are only distantly related to these clades. If future work supports specific status for *quadramaculatus* A, the junior synonym *amphileu-cus* (Bishop 1941) is an available name.

marmoratus B

Kozak *et al.* (2005b) referred to this clade as *quadramaculatus/marmoratus* B, but it appears to consist entirely of salamanders referable to *Desmognathus marmoratus*. Martof (1956) described populations of *D. marmoratus* from the Apalachicola drainage as a subspecies, *D. m. aureatus*, and populations from the Savannah drainage as another subspecies, *D. m. roboratus*; both subspecific epithets represent available names for this clade.

quadramaculatus/marmoratus C

Pope (1928) described a population of *Desmognathus marmoratus* from the Pigeon River drainage near Waynesville, North Carolina, as the subspecies *D. m. intermedia*, which was subsequently elevated to full specific status (Pope & Hairston 1951). In turn, Martof (1956) relegated *D. intermedia* to subspecific status when he described a new subspecies, *D. m. meliana*, from the Nantahala River drainage, only to later reject subspecific status for all populations of *D. marmoratus* (Martof 1962). Thus, both *intermedia* and *melania* are available names, but further genetic characterization as well as investigation into interactions between the *quadramaculatus*-like and *marmoratus*-like populations that comprise this clade must necessarily precede any nomenclatural change.

quadramaculatus D

Kozak *et al.* (2005b) referred to this clade as *quadramaculatus/marmoratus* D, but it appears to consist entirely of salamanders referable to *Desmognathus quadramaculatus*.

quadramaculatus/marmoratus E

This clade fits the pattern described by Kozak *et al.* (2005b) and Jones *et al.* (2006) where populations of *Desmognathus quadramaculatus* and *D. marmoratus* within the same drainage are more closely related to each other than to conspecifics from other drainages. Jones *et al.* (2006) considered the absence of morphologically intermediate populations to be evidence against contemporary hybridization. Salamanders in this clade, matching the morphological descriptions of *D. quadramaculatus* and *D. marmoratus*, co-occur at the type locality of *D. marmoratus*.

quadramaculatus F

Schmidt (1953) restricted the type locality of *Desmognathus quadramaculatus* to the North Carolina side of the Great Smoky Mountains. If his type locality restriction were to be accepted, then the useage of *D. quadramaculatus* should be restricted to the *quadramaculatus* F lineage. However, he had no authority to restrict the type locality in this manner and the correct assignment of the binomial *Desmognathus quadramaculatus* remains a matter of uncertainty because a specific type locality was not given.

Desmognathus ocoee

Dusky salamanders currently assigned to Desmognathus ocoee consist of several genetically distinct populations (Kozak et al. 2005b; Tilley & Mahoney 1996). Moreover, clades comprising *D. ocoee* are only distantly related to an informal assemblage called the *D. ochrophaeus* complex, to which this species had been assigned. Thus we argue that *D. ocoee* should no longer be considered a member of the *ochrophaeus* complex.

We designate two informal groups to reflect relationships among the eight clades that compose *Desmognathus ocoee*. The first group, termed the *ocoee* complex, contains the clades *ocoee* E, *ocoee* F, *ocoee* G and *ocoee* H, and is so named because it includes topotypic material for *D. ocoee*. The second group, the *perlapsus* complex, contains clades *ocoee* A, *ocoee* B, *ocoee* C and *ocoee* D, and is named for topotypic material for *perlapsus*, which is nested within *ocoee* D. These informal taxonomic partitions underscore the marked genetic divergence observed between the two complexes.

Desmognathus apalachicolae

As is the case for the *ocoee* clades, *Desmognathus apalachicolae* is also distantly related to other mountain dusky salamanders and therefore should no longer be considered a member of the *ochrophaeus* complex.

Desmognathus monticola

In general, populations of the geographically widespread species *Desmognathus monticola* are characterized by genetic homogeneity. Topotypic material for *D. monticola* falls within the *monticola* B clade. The subspecies *D. m. jeffersoni*, described by Hoffman (1951), has not been recognized in recent treatments. Topotypic samples for *D. m. jeffersoni* are also deeply nested within the *monticola* B clade; therefore, the epithet *jeffersoni* is not an available name for either the *monticola* A or C clades. Populations of the *monticola* C clade tend to be more darkly pigmented, particularly ventrally, and Rubenstein (1969) stated that this form was to be described as a new species but the description was never published.

Desmognathus carolinensis

Desmognathus carolinensis is yet another species considered to be a member of a mountain dusky (*D. ochrophaeus*) complex on the basis of morphology and occasional hybridization with *D. orestes* (Mead *et al.* 2001; Tilley & Mahoney 1996). Like the aforementioned *D. ocoee* and *D. apalachicolae*, *D. carolinensis* is only distantly related to *D. ochrophaeus* and should be removed from the *ochrophaeus* complex.

Desmognathus abditus

When first described, *D. abditus* was considered to be a member of the *D. ochrophaeus* complex on the basis of morphology and occasional hybridization with *D. ochrophaeus* (Anderson & Tilley 2003). However, *D. abditus* was not recovered as a member of the clade that includes *D. ochrophaeus* and, furthermore, appears to be only distantly related to the clade. Thus, *D. abditus* should no longer be considered part of the *ochrophaeus* complex.

Desmognathus conanti complex

Desmognathus conanti was originally described as a subspecies of *D. fuscus* (Rossman 1958), an arrangement followed until Titus & Larson (1996) demonstrated that the two taxa are not closely related. Although Bonett (2002) documented hybridization between *D. conanti* and *D. fuscus* in western Kentucky, it is limited; thus, he recognized *D. conanti* as a distinct species.

Given the corroborative support for specific status of *Desmognathus conanti* (Beamer & Lamb 2008; Kozak *et al.* 2005b) and its distant relationship to *D. fuscus*, this species should no longer be included in the *D. fuscus* complex. Instead, we propose an informal taxonomic grouping to be known as the *conanti* complex, which would include the lineages *conanti* A, *conanti* B, *conanti* C, *conanti* D, and *conanti* E from this study, the β clade and γ clade from Tilley *et al.*(2013), and *D. santeetlah*. The *D. conanti* complex is defined by genealogical exclusivity of haplotypes, morphological similarity, parapatric distributions, and varying levels of gene flow between certain clades.

The range extent of this species complex does not differ significantly from previously published distributions (Fig. 40), though we provide some clarification regarding the northeastern range extent. Beamer and Lamb (2008) published a range revision for *Desmognathus conanti* that showed its distribution extending into southwestern Virginia. However, Tilley *et al.* (2013) countered that no individuals of *D. conanti* had ever been collected in Virginia and, based on their sampling from areas nearby in Tennessee, concluded the species does not occur in Virginia. This survey confirms the absence of *D. conanti* from that area in Virginia; *D. conanti* does not appear to occur north of the French Broad River valley (Tilley *et al.* 2013).

auriculatus A

Dusky salamander populations throughout the Atlantic and Gulf coastal plains have been referred to *Desmognathus auriculatus* (Means 1974; Petranka 1998), but Beamer and Lamb (2008) demonstrated that many coastal plain populations are in fact referable to other species. In this study, we recovered two genealogically exclusive though topologically disparate clades matching the morphological description of *D. auriculatus*. We assign just one, *auriculatus* A, to represent *D. auriculatus*.

Desmognathus fuscus complex

Above, we removed *Desmognathus santeetlah* and all clades within *D. conanti* from an informal taxonomic group traditionally recognized as the *fuscus* complex. Here we redefine the *fuscus* complex to better reflect its evolutionary history; it should include the clades *D. planiceps, fuscus* A, B, C and D, as well as *auriculatus* B and C.

The *fuscus* complex is defined by genealogical exclusivity of haplotypes, morphological similarity, and parapatric distributions.

Desmognathus ochrophaeus complex

Desmognathus imitator; D. abditus, D. apalachicolae and members of the ocoee and perlapsus complexes have all been removed from the informal taxonomic group known as the ochrophaeus complex. Here we reconfigure the ochrophaeus complex to include the clades D. ochrophaeus, orestes A, orestes B and orestes C, which are all closely related and morphologically similar.

Future directions. The clades defined and species complex rearrangements presented herein offer a much improved framework for future work on the phylogenetics and taxonomy of *Desmognathus*. Analyses of our mtDNA dataset proved highly effective in not only recovering clades but offering important insight to relationships. However, many otherwise well-supported clades are separated by short branches, likely reflecting the broadscale adaptive radiation reported for this genus following the evolution of a larval stage (Chippindale *et al.* 2004; Kozak *et al.* 2005b). Limited existing genomic resources (and the exceptionally large genomes of salamanders) hampered our efforts to secure appropriate nuclear DNA sequence data. Newly developed methodology should help overcome this hurdle (Lemmon *et al.* 2012), and one of us (DAB) has begun to generate genomic data that appear suitable for resolving otherwise short, poorly supported branches in the mtDNA phylogeny. Satisfactory resolution of the systematic vagaries that have characterized this genus for so long may soon be achieved.

Acknowledgements

We thank the anonymous reviewers for their helpful reviews of this manuscript. Steve Bennett, Sean Graham, Matthew Beamer, Denise Beamer, Jon Davenport, Paul Moler, Dirk Stevenson, Jeff Boundy, Wayne Van Devender, J.D. Kleopfer, Mike Ross, Brian Duracka, Emily Moriarty Lemmon, Jeff Hall, Ed Corey, Alvin Braswell, Brent Hendrixson, Paul Marek, Matt Walker, and Jessica Wooten provided help in the field and/or useful specimens. Alan Resetar, Travis LaDuc, Addison Wynne, Alvin Braswell and Toby Hibbits provided access and/or useful information with museum specimens. Jason Bond provided access and help with computing resources for analysis. This research was supported in part by a Theodore Roosevelt Memorial Grant from the American Museum of Natural History, a grant from the North Carolina Herpetological Society, a State Wildlife Grant from South Carolina and National Science Foundation—DEB-0808451.

References

- Anderson, J.A. & Tilley, S.G. (2003) Systematics of the *Desmognathus ochrophaeus* complex in the Cumberland Plateau of Tennessee. *Herpetological Monographs*, 17, 75–110.
 - https://doi.org/10.1655/0733-1347(2003)017[0075:SOTDOC]2.0.CO;2
- Barbour, R.W. (1950) A new subspecies of the salamander *Desmognathus fuscus*. *Copeia*, 1950, 277–278. https://doi.org/10.2307/1437906
- Bazinet, A.L., Zwickl, D.J. & Cummings, M.P. (2014) A gateway for phylogenetic analysis powered by grid computing featuring GARLI 2.0. Systematic Biology, 63, 812–818. https://doi.org/10.1093/sysbio/syu031
- Beamer, D.A. & Lamb, T. (2008) Dusky salamanders (*Desmognathus*, Plethodontidae) from the Coastal Plain: Multiple independent lineages and their bearing on the molecular phylogeny of the genus. *Molecular Phylogenetics and Evolution*, 47, 143–153.

https://doi.org/10.1016/j.ympev.2008.01.015

Bingham, R.E., Papenfuss, T.J., Lindstrand, L. & Wake, D.B. (2018) Phylogeography and species boundaries in the *Hydroman*tes shastae complex, with description of two new species (Amphibia; Caudata; Plethodontidae). Bulletin of the Museum of Comparative Zoology, 161, 403–427.

https://doi.org/10.3099/MCZ42.1

- Bishop, S.C. (1941) Notes on salamanders with descriptions of several new forms. Occasional Papers of the Museum of Zoology, 451, 1–21.
- Bishop, S.C. & Valentine, B.D. (1950) A new species of Desmognathus from Alabama. Copeia, 1950, 39-43.

https://doi.org/10.2307/1437580

- Bonett, R.M. (2002) Analysis of the contact zone between the dusky salamanders *Desmognathus fuscus fuscus and Desmogna-thus fuscus conanti* (Caudata: Plethodontidae). *Copeia*, 2002, 344–355. https://doi.org/10.1643/0045-8511(2002)002[0344:AOTCZB]2.0.CO:2
- Bonett, R.M., Kozak, K.H., Vieites, D.R., Bare, A., Wooten, J.A. & Trauth, S.E. (2007) The importance of comparative phylogeography in diagnosing introduced species: a lesson from the seal salamander, *Desmognathus monticola*. *BMC Ecology*, 7, 7.

https://doi.org/10.1186/1472-6785-7-7

Bruce, R.C. (1991) Evolution of ecological diversification in desmognathine salamanders. Herpetological Review, 22, 44-45.

Camp, C.D., Peterman, W.E., Milanovich, J.R., Lamb, T., Maerz, J.C. & Wake, D.B. (2009) A new genus and species of lungless salamander (family Plethodontidae) from the Appalachian highlands of the south-eastern United States. *Journal of Zoology*, 279, 86–94.

https://doi.org/10.1111/j.1469-7998.2009.00593.x

- Camp, C.D., Tilley, S.G., Austin Jr, R.M. & Marshall, J.L. (2002) A new species of black-bellied salamander (genus *Desmogna-thus*) from the Appalachian Mountains of northern Georgia. *Herpetologica*, 58, 471–484. https://doi.org/10.1655/0018-0831(2002)058[0471:ANSOBS]2.0.CO;2
- Chippindale, P.T., Bonett, R.M., Baldwin, A.S. & Wiens, J.J. (2004) Phylogenetic evidence for a major reversal of life-history evolution in plethodontid salamanders. *Evolution*, 58, 2809–2822. https://doi.org/10.1111/j.0014-3820.2004.tb01632.x
- Conant, R. & Collins, J.T. (1998) A Field Guide to Reptiles & Amphibians: Eastern and Central North America. Houghton Mifflin, 3rd edition, 640 pp.
- Crespi, E.J., Browne, R.A. & Rissler, L.J. (2010) Taxonomic revision of *Desmognathus wrighti* (Caudata: Plethodontidae). *Herpetologica*, 66, 283–295.

https://doi.org/10.1655/HERPETOLOGICA-D-09-00002.1

- Dodd, C.K. (2004) The Amphibians of Great Smoky Mountains National Park. University of Tennessee Press. Available from: https://books.google.com/books?id=5ECpg6ilRSQC (accessed 20 April 2019) https://doi.org/10.3133/cir1258
- Edgar, R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 32, 1792–1797.

https://doi.org/10.1093/nar/gkh340

- Edwards, S.V. (2009) Is a new and general theory of molecular systematics emerging? *Evolution*, 63, 1–19. https://doi.org/10.1111/j.1558-5646.2008.00549.x
- Fenneman, N.M. (1938) Physiography of eastern United States. McGraw-Hill Book Company, inc.
- Frost, D.R. (2014) *Amphibian Species of the World: an Online Reference*. American Museum of Natural History, New York. Available from: http://research.amnh.org/herpetology/amphibia/index.html (accessed 20 April 2019)
- García-París, M., Good, D.A., Parra-Olea, G. & Wake, D.B. (2000) Biodiversity of Costa Rican salamanders: implications of high levels of genetic differentiation and phylogeographic structure for species formation. *Proceedings of the National Academy of Sciences*, 97, 1640–1647.

https://doi.org/10.1073/pnas.97.4.1640

- García-París, M., Parra-Olea, G. & Wake, D.B. (2008) Description of a new species of the *Bolitoglossa subpalmata* group (Caudata: Plethodontidae) from Costa Rica. *The Herpetological Journal*, 18, 23–31.
- Graham, S.P., Beamer, D. & Lamb, T. (2012) Good news at last: conservation status of the seepage salamander (*Desmognathus aeneus*). *Herpetological Conservation and Biology*, 7, 339–348.
- Graham, S.P., Timpe, E.K. & Laurencio, L.R. (2010) Status and possible decline of the Southern Dusky Salamander (*Desmognathus auriculatus*) in Georgia and Alabama, USA. *Herpetological Conservation and Biology*, 5, 360–373.
- Graziano, M. & Reid, M.L. (2006) An addition to the herpetofauna of Ohio. Journal of Kansas Herpetology, 17, 6.

Hairston, N.G. (1949) The local distribution and ecology of the plethodontid salamanders of the Southern Appalachians. *Ecological Monographs*, 19, 47–73. https://doi.org/10.2307/1943584

- Hairston, N.G. (1987) *Community ecology and salamander guilds*. Cambridge University Press. Available from: https://books.google.com/books?id=zyLpHnT0CfcC (accessed 20 April 2019)
- Hamilton, C.A., Hendrixson, B.E., Brewer, M.S. & Bond, J.E. (2014) An evaluation of sampling effects on multiple DNA barcoding methods leads to an integrative approach for delimiting species: A case study of the North American tarantula genus *Aphonopelma* (Araneae, Mygalomorphae, Theraphosidae). *Molecular Phylogenetics and Evolution*, 71, 79–93. https://doi.org/10.1016/j.ympev.2013.11.007
- Hebert, P. & Gregory, T.R. (2005) The promise of DNA barcoding for taxonomy. *Systematic Biology*, 54, 852–859. https://doi.org/10.1080/10635150500354886
- Hendrixson, B.E. & Bond, J.E. (2005) Testing species boundaries in the Antrodiaetus unicolor complex (Araneae: Mygalomorphae: Antrodiaetidae): "Paraphyly" and cryptic diversity. Molecular Phylogenetics and Evolution, 36, 405–416. https://doi.org/10.1016/j.ympev.2005.01.021
- Hibbitts, T.J., Wahlberg, S.A. & Voelker, G. (2015) Resolving the identity of Texas Desmognathus. Southeastern Naturalist, 14,

213-220.

https://doi.org/10.1656/058.014.0206

Highton, R. (1979) A new cryptic species of salamander of the genus *Plethodon* from the southeastern United States (Amphibia: Plethodontidae). *Brimleyana*, 1, 33–36.

- Highton, R. (1989) Biochemical evolution in the slimy salamanders of the *Plethodon glutinosus* complex in the eastern United States—Part I: Geographic protein variation. *Illinois Biological Monographs*, 57, 1–78, 93–144. https://doi.org/10.5962/bhl.title.49905
- Highton, R. (1999) Geographic protein variation and speciation in the salamanders of the *Plethodon cinereus* group with the description of two new species. *Herpetologica*, 43–90.
- Highton, R. (2014) Detecting cryptic species in phylogeographic studies: Speciation in the California Slender Salamander, *Batrachoseps attenuatus*. *Molecular Phylogenetics and Evolution*, 71, 127–141. https://doi.org/10.1016/j.ympev.2013.11.002
- Highton, R. & Peabody, R.B. (2000) Geographic protein variation and speciation in salamanders of the *Plethodon jordani* and *Plethodon glutinosus* complexes in the southern Appalachian Mountains with the description of four new species. In: Bruce, R.C., Jaeger, R.G. & Houck, L.D. (Eds.), *The Biology of Plethodontid Salamanders*. Kluwer Academic/Plenum Publishers, New York, New York, pp. 31–93. https://doi.org/10.1007/978-1-4615-4255-1 3
- Hoffman, R.L. (1951) A new subspecies of salamander from Virginia. Journal of the Elisha Mitchell Scientific Society, 67, 249–253.
- Jacobs, J.F. (1987) A preliminary investigation of geographic genetic variation and systematics of the two-lined salamander, *Eurycea bislineata* (Green). *Herpetologica*, 43, 423–446.
- Jensen, J.B., Camp, C.D., Gibbons, W. & Elliot, M.J. (2008) *Amphibians and Reptiles of Georgia*. University of Georgia Press, 575 pp.
- Jockusch, E.L., Martinez-Solano, I., Hansen, R.W. & Wake, D.B. (2012) Morphological and molecular diversification of slender salamanders (Caudata: Plethodontidae: *Batrachoseps*) in the southern Sierra Nevada of California with descriptions of two new species. *Zootaxa*, 3190, 1–30.

https://doi.org/10.11646/zootaxa.3190.1.1

- Jockusch, E.L. & Wake, D.B. (2002) Falling apart and merging: diversification of slender salamanders (Plethodontidae: *Batra-choseps*) in the American West. *Biological Journal of the Linnean Society*, 76, 361–391. https://doi.org/10.1111/j.1095-8312.2002.tb01703.x
- Jockusch, E.L., Yanev, K.P. & Wake, D.B. (2001) Molecular phylogenetic analysis of slender salamanders, genus *Batrachoseps* (Amphibia: Plethodontidae), from central coastal California with descriptions of four new species. *Herpetological Monographs*, 15, 54–99.

https://doi.org/10.2307/1467038

- Jones, M.T., Voss, S.R., Ptacek, M.B., Weisrock, D.W. & Tonkyn, D.W. (2006) River drainages and phylogeography: An evolutionary significant lineage of shovel-nosed salamander (*Desmognathus marmoratus*) in the southern Appalachians. *Molecular Phylogenetics and Evolution*, 38, 280–287. https://doi.org/10.1016/j.ympev.2005.05.007
- Karlin, A.A. & Guttman, S.I. (1981) Hybridization between *Desmognathus fuscus* and *Desmognathus ochrophaeus* in northeastern Ohio and northwestern Pennsylvania. *Copeia*, 1981, 371–377.
- Karlin, A.A. & Guttman, S.I. (1986) Systematics and geographic isozyme variation in the plethodontid salamander *Desmognathus fuscus* (Rafinesque). *Herpetologica*, 42, 281–301.
- Karlin, A.A., Guttman, S.I. & Means, D.B. (1993) Population structure in the Ouachita Mountain dusky salamander: *Desmognathus brimleyorum* (Caudata: Plethodontidae). *Southwestern Naturalist*, 38, 36–42. https://doi.org/10.2307/3671642
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, A., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duncan, C., Thierer, T., Ashton, B., Mentjies, P. & Drummond, A. (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28, 1647–1649. https://doi.org/10.1093/bioinformatics/bts199
- Kozak, K.H., Blaine, R.A. & Larson, A. (2005)a) Gene lineages and eastern North American palaeodrainage basins: phylogeography and speciation in salamanders of the *Eurycea bislineata* species complex. *Molecular Ecology*, 15, 191–207. https://doi.org/10.1111/j.1365-294X.2005.02757.x
- Kozak, K.H., Larson, A., Bonett, R.M. & Harmon, L.J. (2005)b) Phylogenetic analysis of ecomorphological divergence, community structure, and diversification rates in dusky salamanders (Plethodontidae: *Desmognathus*). Evolution, 59, 2000– 2016.

https://doi.org/10.1111/j.0014-3820.2005.tb01069.x

- Kozak, K.H., Mendyk, R.W. & Wiens, J.J. (2009) Can parallel diversification occur in sympatry? Repeated patterns of body-size evolution in coexisting clades of North American salamanders. *Evolution*, 63, 1769–1784. https://doi.org/10.1111/j.1558-5646.2009.00680.x
- Lemmon, A.R., Emme, S.A. & Lemmon, E.M. (2012) Anchored hybrid enrichment for massively high-throughput phylogenomics. *Systematic Biology*, 61, 727–744.

https://doi.org/10.1093/sysbio/sys049

- Lemmon, E.M., Lemmon, A.R., Collins, J.T., Lee-Yaw, J.A. & Cannatella, D.C. (2007) Phylogeny-based delimitation of species boundaries and contact zones in the trilling chorus frogs (*Pseudacris*). *Molecular Phylogenetics and Evolution*, 44, 1068–1082.
- https://doi.org/10.1016/j.ympev.2007.04.010
- Maddison, W.P. (1997) Gene trees in species trees. Systematic Biology, 46, 523-526.
- https://doi.org/10.1093/sysbio/46.3.523
- Marek, P.E. (2010) A revision of the Appalachian millipede genus *Brachoria* Chamberlin, 1939 (Polydesmida: Xystodesmidae: Apheloriini). *Zoological Journal of the Linnean Society*, 159, 817–889. https://doi.org/10.1111/j.1096-3642.2010.00633.x
- Martínez-Solano, I., Jockusch, E.L. & Wake, D.B. (2007) Extreme population subdivision throughout a continuous range: phylogeography of *Batrachoseps attenuatus* (Caudata: Plethodontidae) in western North America. *Molecular Ecology*, 16, 4335–4355.

https://doi.org/10.1111/j.1365-294X.2007.03527.x

- Martínez-Solano, Í., Peralta-García, A., Jockusch, E.L., Wake, D.B., Vázquez-Domínguez, E. & Parra-Olea, G. (2012) Molecular systematics of *Batrachoseps* (Caudata, Plethodontidae) in southern California and Baja California: Mitochondrialnuclear DNA discordance and the evolutionary history of *B. major. Molecular Phylogenetics and Evolution*, 63, 131–149. https://doi.org/10.1016/j.ympev.2011.12.026
- Martof, B.S. (1956) *Three new subspecies of Leurognathus marmorata from the Southern Appalachian Mountains*. Occasional papers of the Museum of Zoology, University of Michigan, 21 pp.
- Martof, B.S. (1962) Some aspects of the life history and ecology of the salamander *Leurognathus*. American Midland Naturalist, 67, 1–35.

https://doi.org/10.2307/2422814

- Matson, T., Pfingsten, R.A., Davic, R.D. & Pucci, T.M. (2010) Reexamination of new state record for *Desmognathus monticola* (Seal Salamander) for Ohio. *Herpetological Review*, 41, 17–18.
- Mead, L.S., Tilley, S.G. & Katz, L.A. (2001) Genetic structure of the Blue Ridge dusky salamander (*Desmognathus orestes*): inferences from allozymes, mitochondrial DNA, and behavior. *Evolution*, 55, 2287–2302. https://doi.org/10.1111/j.0014-3820.2001.tb00743.x
- Means, D.B. (1974) The status of *Desmognathus brimleyorum* Stejneger and an analysis of the genus *Desmognathus* in Florida. *Bulletin of the Florida State Museum, Biological Sciences,* 18, 1–100.
- Means, D.B. & Karlin, A.A. (1989) A new species of *Desmognathus* from the eastern Gulf Coastal Plain. *Herpetologica*, 45, 37-46.
- Means, D.B., Lamb, J.Y. & Bernardo, J. (2017) A new species of dusky salamander (Amphibia: Plethodontidae: *Desmognathus*) from the Eastern Gulf Coastal Plain of the United States and a redescription of *D. auriculatus*. *Zootaxa* 4263, 467–506.
- Means, D.B. & Travis, J. (2007) Declines in ravine-inhabiting dusky salamanders of the southeastern US Coastal Plain. Southeastern Naturalist, 6, 83–96.

https://doi.org/10.1656/1528-7092(2007)6[83:DIRDSO]2.0.CO;2

- Mueller, R.L., Macey, J.R., Jaekel, M., Wake, D.B. & Boore, J.L. (2004) Morphological homoplasy, life history evolution, and historical biogeography of plethodontid salamanders inferred from complete mitochondrial genomes. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 13820–13825. https://doi.org/10.1073/pnas.0405785101
- Nylander, J. (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University 2.
- Organ, J.A. (1961) Studies of the local distribution, life history, and population dynamics of the salamander genus *Desmogna-thus* in Virginia. *Ecological Monographs*, 31, 189–220. https://doi.org/10.2307/1950754
- Parra-Olea, G. & Wake, D.B. (2001) Extreme morphological and ecological homoplasy in tropical salamanders. *Proceedings of the National Academy of Sciences*, 98, 7888–7891. https://doi.org/10.1073/pnas.131203598
- Petranka, J. (1998) Salamanders of the United States and Canada. Smithsonian Institution Press, Washington, 587 pp.
- Pope, C.H. (1928) Some plethodontid salamanders from North Carolina and Kentucky, with the description of a new race of *Leurognathus. American Museum Novitates*, 306, 1–19hai.
- Pope, C.H. & Hairston, N.G. (1951) The distribution of *Leurognathus*: a southern Appalachian genus of salamanders. *Fieldiana*: *Zoology*, 31, 155–162.
- Rafinesque, C.S. (1820) Annals of nature: or annual synopsis of new genera and species of animals, plants, &c. discovered in North America. TJ Fitzpatrick. ???page numbers??? https://doi.org/10.5962/bhl.title.106763
- Rissler, L.J. & Taylor, D.R. (2003) The phylogenetics of desmognathine salamander populations across the southern Appalachians. *Molecular Phylogenetics and Evolution*, 27, 197–211.

https://doi.org/10.1016/S1055-7903(02)00405-0

Rissler, L.J., Wilbur, H.M. & Taylor, D.R. (2004) The influence of ecology and genetics on behavioral variation in salamander populations across the Eastern Continental Divide. *The American Naturalist*, 164, 201–213.

https://doi.org/10.1086/422200

Ronquist, F. & Huelsenbeck, J.P. (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574.

https://doi.org/10.1093/bioinformatics/btg180

Rossman, D.A. (1958) A new race of *Desmognathus fuscus* from the south-central United States. *Herpetologica*, 14, 158–160.

Rovito, S.M., Parra-Olea, G., Recuero, E. & Wake, D.B. (2015) Diversification and biogeographical history of Neotropical plethodontid salamanders: Neotropical salamander diversification. *Zoological Journal of the Linnean Society*, 175, 167–188.

https://doi.org/10.1111/zoj.12271

Rubenstein, N.M. (1969) A study of the salamanders of Mount Cheaha, Cleburne County, Alabama. *Journal of Herpetology*, 3, 33.

https://doi.org/10.2307/1563222

Rubenstein, N.M. (1971) Ontogenetic allometry in the salamander genus *Desmognathus*. American Midland Naturalist, 85, 329-348.

https://doi.org/10.2307/2423761

- Schmidt, K.P. (1953) *A Check List of North American Amphibians and Reptiles*. 6th ed. American Society of Ichthyologists and Herpetologists and University of Chicago Press., Chicago, U.S.A., 680 pp.
- Sites, J.W. & Crandall, K.A. (1997) Testing species boundaries in biodiversity studies. *Conservation Biology*, 11, 1289–1297. https://doi.org/10.1046/j.1523-1739.1997.96254.x
- Steffen, M.A., Irwin, K.J., Blair, A.L. & Bonett, R.M. (2014) Larval masquerade: a new species of paedomorphic salamander (Caudata: Plethodontidae: *Eurycea*) from the Ouachita Mountains of North America. *Zootaxa*, 3786, 423. https://doi.org/10.11646/zootaxa.3786.4.2
- Tilley, S.G. (1988) Hybridization between two species of *Desmognathus* (Amphibia: Caudata: Plethodontidae) in the Great Smoky Mountains. *Herpetological Monographs*, 2, 27–39. https://doi.org/10.2307/1467025
- Tilley, S.G. (2000) The systematics of *Desmognathus imitator*. *In:* Bruce, R.C., Jaeger, R.G. & Houck, L.D. (Eds.), *The Biology of Plethodontid Salamanders*. Springer US, pp. 121–147. https://doi.org/10.1007/978-1-4615-4255-1_5
- Tilley, S.G. (2016) Patterns of genetic differentiation in woodland and dusky salamanders. *Copeia*, 104, 8–20. https://doi.org/10.1643/OT-15-340
- Tilley, S.G., Bernardo, J., Katz, L.A., López, L., Devon Roll, J., Eriksen, R.L., Kratovil, J., Bittner, N.K.J. & Crandall, K.A. (2013) Failed species, innominate forms, and the vain search for species limits: cryptic diversity in dusky salamanders (*Desmognathus*) of eastern Tennessee. *Ecology and Evolution*, 3, 2547–2567. https://doi.org/10.1002/ece3.636
- Tilley, S.G., Eriksen, R.L. & Katz, L.A. (2008) Systematics of dusky salamanders, *Desmognathus* (Caudata: Plethodontidae), in the mountain and Piedmont regions of Virginia and North Carolina, USA. *Zoological Journal of the Linnean Society*, 152, 115–130.

https://doi.org/10.1111/j.1096-3642.2007.00336.x

- Tilley, S.G., Highton, R. & Wake, D.B. (2012) Caudata—Salamanders. *In:* Crother, B.I. (Ed.), *Scientific and standard English* names of amphibians and reptiles of North America north of Mexico, with comments regarding confidence in our understanding. Herpetological Circular No. 29, Society for the Study of Reptiles and Amphibians.
- Tilley, S.G. & Mahoney, M.J. (1996) Patterns of genetic differentiation in salamanders of the *Desmognathus ochrophaeus* complex (Amphibia: Plethodontidae). *Herpetological Monographs*, 10, 1–42. https://doi.org/10.2307/1466979
- Tilley, S.G., Merritt, R.B., Wu, B. & Highton, R. (1978) Genetic differentiation in salamanders of the *Desmognathus ochropha-eus* complex (Plethodontidae). *Evolution*, 32, 93–115. https://doi.org/10.2307/2407413
- Tilley, S.G. & Schwerdtfeger, P.M. (1981) Electrophoretic variation in Appalachian populations of the *Desmognathus fuscus* complex (Amphibia: Plethodontidae). *Copeia*, 1981, 109–119. https://doi.org/10.2307/1444045
- Timpe, E.K., Graham, S.P. & Bonett, R.M. (2009) Phylogeography of the brownback salamander reveals patterns of local endemism in Southern Appalachian springs. *Molecular Phylogenetics and Evolution*, 52, 368–376. https://doi.org/10.1016/j.ympev.2009.03.023
- Titus, T.A. & Larson, A. (1996) Molecular phylogenetics of desmognathine salamanders (caudata: plethodontidae): a reevaluation of evolution in ecology, life history, and morphology. *Systematic Biology*, 45, 451–472. https://doi.org/10.1093/sysbio/45.4.451
- Trauth, S.E., Robinson, H.W. & Plummer, M.V. (2004) *The Amphibians and Reptiles of Arkansas (p)*. University of Arkansas Press, 440 pp.
- Valentine, B.D. (1961) Variation and distribution of *Desmognathus ocoee* Nicholls (Amphibia: Plethodontidae). *Copeia*, 315–322.

https://doi.org/10.2307/1439806

- Valentine, B.D. (1963) The salamander genus *Desmognathus* in Mississippi. *Copeia*, 1963, 130–139. https://doi.org/10.2307/1441280
- Vieites, D.R., Min, M.-S. & Wake, D.B. (2007) Rapid diversification and dispersal during periods of global warming by plethodontid salamanders. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 19903–19907. https://doi.org/10.1073/pnas.0705056104
- Voss, S.R., Smith, D.G., Beachy, C.K. & Heckel, D.G. (1995) Allozyme variation in neighboring isolated populations of the plethodontid salamander *Leurognathus marmoratus*. *Journal of Herpetology*, 29, 493–497. https://doi.org/10.2307/1565011
- Wake, D.B. (1991) Homoplasy: the result of natural selection, or evidence of design limitations? *American Naturalist*, 138, 543–567.

https://doi.org/10.1086/285234

Wake, D.B. (2009) What salamanders have taught us about evolution. *Annual Review of Ecology, Evolution, and Systematics,* 40, 333–352.

https://doi.org/10.1146/annurev.ecolsys.39.110707.173552

- Walker, M.J., Stockman, A.K., Marek, P.E. & Bond, J.E. (2009) Pleistocene glacial refugia across the Appalachian Mountains and coastal plain in the millipede genus *Narceus*: Evidence from population genetic, phylogeographic, and paleoclimatic data. *BMC Evolutionary Biology*, 9, 25. https://doi.org/10.1186/1471-2148-9-25
- Weisrock, D.W., Kozak, K.H. & Larson, A. (2005) Phylogeographic analysis of mitochondrial gene flow and introgression in the salamander, *Plethodon shermani*. *Molecular Ecology*, 14, 1457–1472. https://doi.org/10.1111/j.1365-294X.2005.02524.x
- Weisrock, D.W. & Larson, A. (2006) Testing hypotheses of speciation in the *Plethodon jordani* species complex with allozymes and mitochondrial DNA sequences. *Biological Journal of the Linnean Society*, 89, 25–51. https://doi.org/10.1111/j.1095-8312.2006.00655.x
- Wiens, J.J. (2007) Global patterns of diversification and species richness in amphibians. *The American Naturalist* 170, S86–S106.

https://doi.org/10.1086/519396

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, 2585–2603.

https://doi.org/10.1111/j.0014-3820.2006.tb01892.x

Wiens, J.J. & Penkrot, T.A. (2002) Delimiting species using DNA and morphological variation and discordant species limits in spiny lizards (*Sceloporus*). *Systematic Biology* 51, 69–91. https://doi.org/10.1080/106351502753475880