Article

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*

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Abstract

The Coastal Plain of the southeastern U. S. is one of the planet's top biodiversity hotspots and yet many taxa have not been adequately studied. The plethodontid salamander, *Desmognathus auriculatus*, was originally thought to occur from east Texas to Virginia, a range spanning dozens of interfluves and large river systems. Beamer and Lamb (2008) found five independent mitochondrial lineages of what has been called *D. auriculatus* in the Atlantic Coastal Plain, but did not examine the extensive distribution of *D. auriculatus* in the Gulf Coastal Plain. We present morphological and molecular genetic data distinguishing two evolutionarily independent and distantly related lineages that are currently subsumed under the taxon *D. auriculatus* in the eastern Gulf Coastal Plain. We describe one of these as a new species, *Desmognathus valentinei* **sp. nov.**, and assign the second one to *D. auriculatus* which we formally redescribe.

Key words: cryptic species; Gulf Coastal Plain, *Desmognathus auriculatus*, *D. valentinei* **sp. nov.**, swamp habitat

Introduction

The Coastal Plain of the southeastern United States is a distinct physiographic (Fenneman 1938) and geologic (Thornbury 1965) province of the North American continent. It is a large, seaward-sloping plain extending 2,200 miles from Cape Cod to the Mexican border, and an additional 1,000 miles along the western side of Mexico. All the major drainages of the eastern side of the North American continent flow across this huge province into either the Atlantic Ocean or Gulf of Mexico. In the United States, the Florida peninsula separates the Atlantic Coastal Plain to the northeast from the Gulf Coastal Plain to the west. Swamps and extensive fluvial wetlands are common near the seacoasts along the entire Atlantic Coastal Plain and west in the Gulf Coastal Plain into the state of Texas for about 100 km before natural aridity drastically alters wetland ecology all the way into Mexico.

One hundred and seventy nine years ago, Holbrook (1838) described the southern dusky salamander, *Desmognathus auriculatus*, based on specimens from "Riceborough, [Liberty County,] in Georgia." This was the first of the southeastern U. S. Coastal Plain species of the endemic North American radiation of dusky salamanders (Genus *Desmognathus*) to be named, and the moniker has since been applied to phenotypically similar, swamp-inhabiting *Desmognathus* populations throughout the Atlantic and Gulf Coastal Plains from eastern North Carolina and South Carolina, through Georgia, Florida, Mississippi and Louisiana, and extending into eastern Texas (review: Petranka 1998; Means 2005).

The Coastal Plain lacks the variation in relief that is often associated with evolutionary lineage diversity (e.g. Wollenburg *et al*. 2008; López-Pujol *et al*. 2011). The possibility that a single species of *Desmognathus* could span the entire region seems feasible in light of the lack of relief, the extensive distribution of swampy habitats across the Coastal Plain, and the apparent morphological similarity of swamp-inhabiting *Desmognathu*s. However, the

Coastal Plain is dissected by numerous large river systems that act as barriers to gene exchange in many aquatic and terrestrial species (Avise *et al.* 1987; Avise 1992). The southeastern U. S. Coastal Plain harbors substantial cryptic evolutionary diversity (sensu Bernardo 2011), and it has recently been proposed as one of the planet's top 35 biodiversity hotspots (Noss *et al.* 2014). In addition to its high plant species richness and endemism, the Coastal Plain supports many diverse vertebrate groups, including the greatest species richness of frogs in the U. S. and Canada, and six of the nine families of salamanders (Noss *et al.* 2014), including the lungless salamanders (Family Plethodontidae).

Plethodontid diversity has historically been understudied in the Coastal Plain (Means 2000). Cryptic lineages have been detected among Coastal Plain populations of *Eurycea* (Harrison and Guttman, 2003, Lamb and Beamer 2012, Wray 2015, Wray *et al.* 2017). *Desmognathus,* another genus with an extensive distribution in both Atlantic and Gulf coastal plains, likely contains a similar level of hidden diversity (Beamer and Lamb 2008, this study). Careful examination of morphological variation among populations putatively assigned to *D. auriculatus* is wanting (but see Valentine 1963), and the hypothesis of a broadly-ranging (eurytopic), monophyletic lineage of *Desmognathus* inhabiting swampy Coastal Plain habitats has never been scrutinized. Consequently, the name *D. auriculatus* may represent a case of taxonomic inertia (sensu Bernardo 2011). Beamer and Lamb (2008) used a portion of the mitochondrial gene Cytochrome Oxidase I (COx I) to demonstrate that Atlantic Coastal Plain populations of putative *D. auriculatus* comprised five lineages. Hibbitts *et al.* (2015) used the same portion of COx I to confirm that extant populations of *Desmognathus* from Texas were not equivalent to Atlantic Coastal Plain populations of *D. auriculatus* from near the type locality. However, neither study included *D. auriculatus* populations from Gulf Coastal Plain drainages west of the Apalachicola River. We address this gap with genetic data from Mississippi and Louisiana for populations of putative *D. auriculatus*, many of which were included in Lamb (2016). These genetic data complement our morphological and ecological analyses across populations originally attributed to *D. auriculatus* from this region. Our data demonstrate that Mississippi and eastern Louisiana populations of putative *D. auriculatus* are not conspecific with topotypic specimens from Georgia and we address the taxonomic implications of our findings.

Materials and methods

Preservation. This study made use of materials collected over a long period of time. Specimens collected in the 20th Century were killed in 30% isopropanol and then laid out on paper toweling and soaked in 10% formalin for at least 48 hours before being transferred to 60% isopropanol for long-term storage. In the 21st Century, salamanders were killed in a solution of 5-10% Benzocaine or 0.2% 2-Phenoxyethanol, tail-tip tissue samples were taken from individuals whose sequence data are reported here, and then voucher specimens were fixed in 10% formalin and stored in 70% ethanol.

Molecular data collection and analyses. We generated novel sequence data for multiple populations and species of *Desmognathus* from the Southeastern United States, with an emphasis on the Gulf Coastal Plain (Table 1, Fig. 1). We extracted genomic DNA using the Blood & Tissue DNEasy* Kit (Quagen Group, Valencia, CA). We used both previously published and novel primers (Table 2) to amplify a portion of two mitochondrial genes, Cytochrome Oxidase I (COx I) and Cytochrome B (CytB), as well as one nuclear gene, Recombination Activating Gene 1 (RAG-1). Standard polymerase chain reaction (PCR) conditions for COx I included 0.5 μL template DNA, 0.1 μL *Taq* polymerase, 0.75 μL of each primer, 2 μL each of 25 mM $MgCl_2$ and 200 μM dNTPs, 2.5 μL of NEB buffer, and nuclease free water to a total volume of 25 μL. Conditions for CytB and RAG-1 were similar except that for CytB we used 1.0 μL template, 0.3 μL *Taq* polymerase, and 2.5 μL of 25 mM $MgCl_2$ per reaction, and for RAG-1 we used 0.15 μL *Taq* polymerase. Standard PCR cycling for both COx I and CytB was as follows: 1 cycle at 95°C for 1 min.; 30 cycles of 95°C, 50°C, and then 72°C for 1 min. each; 1 cycle at 72°C for 3 min. PCR cycling for RAG-1 followed Bonett *et al.* (2014). We cleaned amplified DNA using 0.25 μL of Shrimp Alkaline Phosphatase and Exonuclease 1 (USB ®), and heated samples to 37 °C for 15 min. followed by 15 min at 85°C to denature the enzymes. Samples were sequenced by Eurofins Scientific © and we used Sequencher ™ ver. 5.1. to check for any premature stop codons, confirm base calls, and align sequence data. Novel sequences have been submitted to GenBank (COx1: KY658976–KY659003; CytB: KY659004–KY659029; RAG-1: KY659030–KY659037).

TABLE 1. Locality and sequence information for *Desmognathus* used in this study. State names are abbreviated. Not all individuals were vouchered and some have not yet been deposited in museum collections (DBM =D. Bruce Means; JYL = Jennifer Y. Lamb; DAB = David. A. Beamer). LSUMZ = Louisiana State University Museum of Zoology; MVZ = Museum of Vertebrate Zoology; MMNS = Mississippi Museum of Natural Science). Locality number corresponds with the maps in Fig. 1 and 4. Mitochondrial haplotype names reflect the taxon name, truncated names of river drainage(s), and unique COx1 haplotype number, followed by the unique CytB haplotype number, where appropriate. Genes are abbreviated under Accession# as reported in the text. Novel sequences generated here were submitted to GenBank and accession numbers are reported for each gene in each individual sequenced.

Species	Locality #	State	County/Parish	Drainage	Accession#	Specimen#	Mitochondrial Haplotype	Source	Lat.	Long.
Amphiuma means	-	*a*			GQ368656	RMB2489	Amphiuma_GQ368656	Zhang & Wake (2009)	-	-
Aneides flavipunctatus	-	CA	Siskiyou	*a*	AY728214	MVZ 219973	Aneides_AY728214	Mueller *et al.* (2004)	-	-
Desmognathus aeneus	-	NC	Graham	Tennessee	EU311716	DAB953	aeneus_EU311716	Beamer & Lamb (2008)	-	-
D. apalachicolae	30	FL	Liberty	Apalachicola	COx1: KY658976; CytB: KY659004	JYL272	apalachicolae_Apalachie_1_1	This study	30.4925	84.902
D. apalachicolae	30	FL	Liberty	Apalachicola	COx1: KY658976; CytB: KY659005	JYL271	apalachicolae_Apalachie_1_2	This study	30.4925	84.902
D. auriculatus	9	FL	Wakula	Ochlockonee	COx1: KY658977	JYL268	auriculatus_Ochlockonee_1	This study	30.1444	84.5745
D. auriculatus	9	FL	Wakula	Ochlockonee	COx1: KY658977; CytB: KY659006; RAG-1: KY659030	JYL269	auriculatus_Ochlockonee_1_1	This study	30.1444	84.5745
D. auriculatus	9	FL	Wakula	Ochlockonee	COx1: KY658978; CytB: KY659007; RAG-1: KY659031	JYL270	auriculatus_Ochlockonee_2_2	This study	30.1444	84.5745
D. auriculatus	10	GA	Liberty	Ogeechee	EU311680	DAB348	auriculatus_EU311680	Beamer & Lamb (2008)	31.843	81.4718
D. auriculatus	11	GA	Clinch	Suwannee	EU311650	DAB1385	auriculatus_EU311650	Beamer & Lamb (2008)	30.9717	82.821
D. auriculatus	12	FL	Baker	Suwannee	EU311681	DAB349	auriculatus_EU311681	Beamer & Lamb (2008)	30.3931	82.3018
D. auriculatus	13	FL	Wakulla	*a*	*b*	BTL239	auriculatus_BTL239	D. Shepard	30.1302	84.3542
D. brimleyorum	-	AR	Ouachita	*a*	*b*	DBS2394	brimleyorum_DBS2394	D. Shepard	-	-
D. brimleyorum	-	AR	Ouachita	*a*	*b*	KJI1160	brimleyorum_KJI1160	D. Shepard	-	-
D. brimleyorum	-	AR	Nevada	*a*	*b*	RMB2327	brimleyorum_RMB2327	D. Shepard	-	-
D. carolinensis	-	NC	Buncombe	Tennessee	EU311642	DAB1105	carolinensis_EU311642	Beamer & Lamb (2008)	-	-
D. carolinensis	-	NC	Yancey	Tennessee	EU311713	DAB946	carolinensis_EU311713	Beamer & Lamb (2008)	-	-

TABLE 1. (Continued)

Species	Locality #	State	County/ Parish	Drainage	Accession#	Specimen#	Mitochondrial Haplotype	Source	Lat.	Long.
D. cf. *auriculatus*	-	NC	Bladen	Pee Dee	EU311655	DAB1485	cf_auriculatus_EU311655	Beamer & Lamb (2008)	-	-
D. cf. *auriculatus*	-	NC	Carteret	White Oak	EU311656	DAB1487	cf_auriculatus_EU311656	Beamer & Lamb (2008)	-	-
D. cf. *auriculatus*	-	NC	Beaufort	Neuse	EU311664	DAB201	cf_auriculatus_EU311664	Beamer & Lamb (2008)	-	-
D. cf. *auriculatus*	-	NC	Craven	Neuse	EU311665	DAB209	cf_auriculatus_EU311665	Beamer & Lamb (2008)	-	-
D. cf. *auriculatus*	-	NC	Craven	Neuse	EU311682	DAB414	cf_auriculatus_EU311682	Beamer & Lamb (2008)	-	-
D. cf. *auriculatus*	-	NC	Edgecombe	Pamlico	EU311683	DAB434	cf_auriculatus_EU311683	Beamer & Lamb (2008)	-	-
D. cf. *auriculatus*	-	NC	Pitt	Pamlico	EU311719	DAB972	cf_auriculatus_EU311719	Beamer & Lamb (2008)	-	-
D. cf. *auriculatus*	-	SC	Colleton	Broad-St. Helena Sound	EU311686	DAB501	cf_auriculatus_EU311686	Beamer & Lamb (2008)	-	-
D. cf. *auriculatus*	-	SC	Berkeley	Santee	EU311695	DAB637	cf_auriculatus_EU311695	Beamer & Lamb (2008)	-	-
D. cf. *auriculatus*	-	NC	New Hanover	Cape Fear	EU311663	DAB1545	cf_auriculatus_EU311663	Beamer & Lamb (2008)	-	-
D. cf. *auriculatus*	-	SC	Calhoun	Santee	EU311669	DAB265	cf_auriculatus_EU311669	Beamer & Lamb (2008)	-	-
D. cf. *auriculatus*	-	SC	Bamberg	Edisto	EU311711	DAB881	cf_auriculatus_EU311711	Beamer & Lamb (2008)	-	-
D. cf. *auriculatus*	-	NC	Pitt	Pamlico	EU311670	DAB290	cf_auriculatus_EU311670	Beamer & Lamb (2008)	-	-
D. cf. *auriculatus*	-	SC	Florence	Pee Dee	EU311707	DAB806	cf_auriculatus_EU311707	Beamer & Lamb (2008)	-	-
D. cf. *auriculatus*	-	NC	Duplin	Cape Fear	EU311653	DAB1478	cf_auriculatus_EU311653	Beamer & Lamb (2008)	-	-
D. cf. *auriculatus*	-	NC	Bladen	Cape Fear	EU311687	DAB508	cf_auriculatus_EU311687	Beamer & Lamb (2008)	-	-
D. cf. *auriculatus*	-	NC	Scotland	Pee Dee	EU311706	DAB782	cf_auriculatus_EU311706	Beamer & Lamb (2008)	-	-
D. cf. *auriculatus*	-	NC	Scotland	Pee Dee	EU311696	DAB638	cf_auriculatus_EU311696	Beamer & Lamb (2008)	-	-
D. *conanti*	5	MS	Forrest	Pascagoula	COx1: KY658985; CytB: KY659009	JYL262	conanti_BlkCrk_4_11	This study	c	c
D. *conanti*	14	LA	Rapides	Red	COx1: KY658997; CytB: KY659021	JYL318	conanti_Red_1_37	This study	31.2912	92.704

TABLE 1. (Continued)

Species	Locality #	State	County/ Parish	Drainage	Accession#	Specimen#	Mitochondrial Haplotype	Source	Lat.	Long.
D. conanti	15	LA	Natchitoches	Red	COx1: KY658998; CytB: KY659020	LSUMZ H-18126	conanti_Red_5_17	This study	31.5761	93.0729
D. conanti	16	LA	Catahoula	Ouachita	COx1: KY658991; CytB: KY659015	JYL319	conanti_Ouachita_1_18	This study	31.8265	91.7542
D. conanti	16	LA	Catahoula	Ouachita	COx1: KY658992; CytB: KY659015	JYL324	conanti_Ouachita_2_18	This study	31.8265	91.7542
D. sp.	17	MS	Jackson	Pascagoula	COx1: KY658993; CytB: KY659016	JYL78	sp_Pasca_1_5	This study	30.5644	88.6257
D. conanti	17	MS	Jackson	Pascagoula	COx1: KY658994; CytB: KY659017	JYL90	conanti_Pasca_4_14	This study	30.5644	88.6257
D. conanti	17	MS	Jackson	Pascagoula	RAG-1: KY659034	JYL95	-	This study	30.5644	88.6257
D. conanti	18	MS	Wilkinson	Clark Creek	COx1: KY658990; CytB: KY659014	JYL121	conanti_LowerMS_Homo_1_6	This study	31.0658	91.5191
D. conanti	19	MS	Forrest	Pascagoula	COx1: KY658989; CytB: KY659013	JYL10	conanti_Leaf_Pasca_1_7	This study	31.2135	89.1682
D. conanti	20	MS	Marion	Pearl	COx1: KY658995; CytB: KY659018	JYL147	conanti_Pearl_1_12	This study	31.3325	89.9479
D. conanti	21	MS	Franklin	Homochitto	COx1: KY658988; CytB: KY659012	JYL295	conanti_Homo_2_35	This study	31.3517	90.8185
D. conanti	22	MS	Lauderdale	Pascagoula	COx1: KY658986; CytB: KY659010; RAG1: KY659033	JYL174	conanti_Chick_3_15	This study	32.227	88.8329
D. conanti	23	MS	Scott	Pearl	COx1: KY658996; CytB: KY659019	JYL442	conanti_Pearl_3_24	This study	32.3615	89.8749
D. conanti	24	MS	Carrol	Yazoo	COx1: KY659000; CytB: KY659024	JYL429	conanti_Yazoo_2_27	This study	33.4934	89.8706
D. conanti	25	MS	Tishomingo	Tennessee	COx1: KY658999; CytB: KY659023	JYL203	conanti_Tenness_2_28	This study	34.6092	88.1763

TABLE 1. (Continued)

Species	Locality #	State	County/ Parish	Drainage	Accession#	Specimen#	Mitochondrial Haplotype	Source	Lat.	Long.
D. conanti	26	MS	Tishomingo	Tennessee	COx1: KY658999; CytB: KY659022	JYL199	conanti_Tenness_2_22	This study	34.6119	88.1986
D. conanti	27	FL	Walton	Choctawhatchee	COx1: KY658987; CytB: KY659011	JYL629	conanti_Choctawhat_2_44	This study	30.4645	85.8638
D. conanti	27	FL	Walton	Choctawhatchee	COx1: KY658987; CytB: KY659011	JYL631	conanti_Choctawhat_2_44	This study	30.4645	85.8638
D. conanti	27	FL	Walton	Choctawhatchee	COx1: KY658987; CytB: KY659011	JYL640	conanti_Choctawhat_2_44	This study	30.4645	85.8638
D. conanti	28	FL	Santa Rosa	Yellow	RAG-1: KY659032	JYL610	-	This study	30.5797	86.8013
D. conanti	29	FL	Santa Rosa	Yellow	COx1: KY659001; CytB: KY659025	JYL267	conanti_Yellow_1_2	This study	30.5078	86.9161
D. conanti	-	TX	Tyler	Neches	b	94726	conanti_94726	Hibbitts et al. (2015)	-	-
D. conanti	-	TX	Newton	Sabine	b	TJH2756	conanti_TJH2756	Hibbitts et al. (2015)	-	-
D. conanti	-	LA	Washington	Pearl	EU311673	DAB323	conanti_EU311673	Beamer & Lamb (2008)	-	-
D. conanti	-	LA	West Feliciana	Bayou Sara	EU311671	DAB321	conanti_EU311671	Beamer & Lamb (2008)	-	-
D. conanti	-	LA	Grant	Red	EU311699	DAB647	conanti_EU311699	Beamer & Lamb (2008)	-	-
D. conanti	-	MS	Amite	Amite	EU311674	DAB324	conanti_EU311674	Beamer & Lamb (2008)	-	-
D. conanti	-	MS	Jasper	Pascagoula	EU311672	DAB322	conanti_EU311672	Beamer & Lamb (2008)	-	-
D. conanti	-	MS	Jasper	Pascagoula	EU311685	DAB438	conanti_EU311685	Beamer & Lamb (2008)	-	-
D. conanti	-	KY	Livingston	Tennessee	EU311667	DAB222	conanti_EU311667	Beamer & Lamb (2008)	-	-
D. conanti	-	AL	Baldwin	Mobile	EU311678	DAB346	conanti_EU311678	Beamer & Lamb (2008)	-	-
D. conanti	-	AL	Lawrence	Mobile	EU311712	DAB922	conanti_EU311712	Beamer & Lamb (2008)	-	-
D. conanti	-	AL	Butler	Escambia	EU311677	DAB345	conanti_EU311677	Beamer & Lamb (2008)	-	-
D. conanti	-	FL	Santa Rosa	Escambia	EU311679	DAB347	conanti_EU311679	Beamer & Lamb (2008)	-	-

TABLE 1. (Continued)

Species	Locality #	State	County/ Parish	Drainage	Accession#	Specimen#	Mitochondrial Haplotype	Source	Lat.	Long.
D. conanti	-	FL	Washington	Choctawhatchee	EU311684	DAB435	conanti_EU311684	Beamer & Lamb (2008)	-	-
D. conanti	-	GA	Richmond	a	b	TJR2470	conanti_TJR2470	D. Shepard	-	-
D. conanti	-	GA	Wayne	Altamaha	EU311709	DAB867	conanti_EU311709	Beamer & Lamb (2008)	-	-
D. conanti	-	GA	Wayne	Altamaha	EU311710	DAB868	conanti_EU311710	Beamer & Lamb (2008)	-	-
D. conanti	-	GA	Effingham	Savannah	EU311651	DAB1387	conanti_EU311651	Beamer & Lamb (2008)	-	-
D. conanti	-	SC	Barnwell	Savannah	EU311668	DAB252	conanti_EU311668	Beamer & Lamb (2008)	-	-
D. conanti	-	NC	Henderson	Tennessee	EU311698	DAB646	conanti_EU311698	Beamer & Lamb (2008)	-	-
D. folkertsi	-	GA	Union	Tennessee	EU311714	DAB949	folkertsi_EU311714	Beamer & Lamb (2008)	-	-
D. fuscus	-	IN	Jefferson	Ohio	EU311639	DAB1036	fuscus_EU311639	Beamer & Lamb (2008)	-	-
D. fuscus	-	KY	Bath	Ohio	EU311640	DAB1039	fuscus_EU311640	Beamer & Lamb (2008)	-	-
D. fuscus	-	MA	Franklin	a	AY728227	MVZ 219973	fuscus_AY728227	Mueller *et al.* (2004)	-	-
D. fuscus	-	NC	Davie	Pee Dee	EU311654	DAB1484	fuscus_EU311654	Beamer & Lamb (2008)	-	-
D. fuscus	-	NC	Montgomery	Pee Dee	EU311657	DAB1488	fuscus_EU311657	Beamer & Lamb (2008)	-	-
D. fuscus	-	NC	Montgomery	Pee Dee	EU311658	DAB1496	fuscus_EU311658	Beamer & Lamb (2008)	-	-
D. fuscus	-	NC	Davidson	Pee Dee	EU311659	DAB1505	fuscus_EU311659	Beamer & Lamb (2008)	-	-
D. fuscus	-	NC	Iredell	Pee Dee	EU311660	DAB1506	fuscus_EU311660	Beamer & Lamb (2008)	-	-
D. fuscus	-	NC	Wilkes	Pee Dee	EU311662	DAB1517	fuscus_EU311662	Beamer & Lamb (2008)	-	-
D. fuscus	-	NC	Watauga	Ohio	EU311689	DAB526	fuscus_EU311689	Beamer & Lamb (2008)	-	-
D. fuscus	-	NC	Caldwell	Santee	EU311702	DAB715	fuscus_EU311702	Beamer & Lamb (2008)	-	-
D. fuscus	-	NC	Burke	Santee	EU311705	DAB755	fuscus_EU311705	Beamer & Lamb (2008)	-	-
D. fuscus	-	NC	Duplin	a	FC13580	b	fuscus_FC13580	D. Shepard	-	-
D. fuscus	-	VA	Bland	Ohio	EU311641	DAB1042	fuscus_EU311641	Beamer & Lamb (2008)	-	-

TABLE 1. (Continued)

Species	Locality #	State	County/Parish	Drainage	Accession#	Specimen#	Mitochondrial Haplotype	Source	Lat.	Long.
D. fuscus	-	VA	Rockingham	Potomac	EU311692	DAB596	fuscus_EU311692	Beamer & Lamb (2008)	-	-
D. fuscus	-	WV	Randolph	Ohio	EU311694	DAB603	fuscus_EU311694	Beamer & Lamb (2008)	-	-
D. marmoratus	-	GA	Rabun	Savannah	EU311645	DAB1336	marmoratus_EU311645	Beamer & Lamb (2008)	-	-
D. marmoratus	-	NC	Caldwell	Santee	EU311701	DAB700	marmoratus_EU311701	Beamer & Lamb (2008)	-	-
D. marmoratus	-	NC	Macon	Tennessee	EU311718	DAB959	marmoratus_EU311718	Beamer & Lamb (2008)	-	-
D. monticola	31	AL	Shelby	Cahaba	COx1: KY659002; CytB: KY659026	JYL649	monticola_Cahaba_1	This study	-	-
D. monticola	-	NC	Transylvania	Tennessee	EU311717	DAB954	monticola_EU311717	Beamer & Lamb (2008)	-	-
D. monticola	-	TN	Monroe	Tennessee	EU311690	DAB571	monticola_EU311690	Beamer & Lamb (2008)	-	-
D. ochrophaeus	-	WV	Randolph	Ohio	EU311693	DAB602	ochrophaeus_EU311693	Beamer & Lamb (2008)	-	-
D. ocoee	-	GA	Lumpkin	Apalachicola	EU311647	DAB1352	ocoee_EU311647	Beamer & Lamb (2008)	-	-
D. ocoee	-	GA	Douglas	Apalachicola	EU311652	DAB1406	ocoee_EU311652	Beamer & Lamb (2008)	-	-
D. ocoee	-	GA	Union	Tennessee	EU311715	DAB951	ocoee_EU311715	Beamer & Lamb (2008)	-	-
D. ocoee	-	NC	Macon	Tennessee	EU311643	DAB1122	ocoee_EU311643	Beamer & Lamb (2008)	-	-
D. orestes	-	NC	Caldwell	Santee	EU311704	DAB739	orestes_EU311704	Beamer & Lamb (2008)	-	-
D. quadramaculatus	32	NC	Macon	Chatooga	COx1: KY659003; CytB: KY659027	JYL652	quadramaculatus_Chatooga_1_1	This study	-	-
D. quadramaculatus	32	NC	Macon	Chatooga	COx1: KY659003; CytB: KY659028	JYL653	quadramaculatus_Chatooga_1_2	This study	-	-
D. quadramaculatus	-	NC	Madison	Tennessee	EU311648	DAB1355	quadramaculatus_EU311648	Beamer & Lamb (2008)	-	-
D. quadramaculatus	-	NC	Madison	Tennessee	EU311649	DAB1356	quadramaculatus_EU311649	Beamer & Lamb (2008)	-	-
D. quadramaculatus	-	NC	Wilkes	Pee Dee	EU311661	DAB1516	quadramaculatus_EU311661	Beamer & Lamb (2008)	-	-

TABLE 1. (Continued)

Species	Locality#	State	County/Parish	Drainage	Accession#	Specimen#	Mitochondrial Haplotype	Source	Lat.	Long.
D. quadramaculatus	-	NC	Macon	Tennessee	EU311691	DAB579	quadramaculatus_EU311691	Beamer & Lamb (2008)	-	-
D. quadramaculatus	-	NC	Caldwell	Santee	EU311700	DAB660	quadramaculatus_EU311700	Beamer & Lamb (2008)	-	-
D. quadramaculatus	-	*a*			KR827018	*b*	-	Martin *et al.* (2015)	-	-
D. quadramaculatus	-	*a*			KR827017	*b*	-	Martin *et al.* (2015)	-	-
D. santeetlah	-	NC	Graham	Tennessee	EU311676	DAB327	santeetlah_EU311676	Beamer & Lamb (2008)	-	-
D. valentinei	1	MS	Madison	Pearl	COx1: KY658983	JYL437	valentinei_Pearl_BlkCrk_1	This study	32.4017	90.0764
D. valentinei	1	MS	Madison	Pearl	COx1: KY658983; RAG-1: KY659035	JYL438	valentinei_Pearl_BlkCrk_1	This study	32.4017	90.0764
D. valentinei	1	MS	Madison	Pearl	COx1: KY658983	JYL439	valentinei_Pearl_BlkCrk_1	This study	32.4017	90.0764
D. valentinei	1	MS	Madison	Pearl	COx1: KY658983	JYL441	valentinei_Pearl_BlkCrk_1	This study	32.4017	90.0764
D. valentinei	2	LA	St. Tammany	Pearl	COx1: KY658981	JYL351	valentinei_Pearl_1	This study	30.5777	89.9274
D. valentinei	3	LA	St. Tammany	Pearl	COx1: KY658983; RAG-1: KY659036	JYL352	valentinei_Pearl_BlkCrk_1	This study	30.567	89.9255
D. valentinei	4	MS	Lamar	Pascagoula	COx1: KY658983	MMNS 19448	valentinei_Pearl_BlkCrk_1	This study	31.2898	89.4502
D. valentinei	4	MS	Lamar	Pascagoula	COx1: KY658983	MMNS 19449	valentinei_Pearl_BlkCrk_1	This study	31.2898	89.4502
D. valentinei	5	MS	Forrest	Pascagoula	COx1: KY658984; CytB: KY659008; RAG-1: KY659037	MMNS 19452	valentinei_Pearl_BlkCrk_2_1	This study	*c*	*c*
D. valentinei	5	MS	Forrest	Pascagoula	COx1: KY658984; CytB: KY659008	JYL265	valentinei_Pearl_BlkCrk_2_1	This study	*c*	*c*
D. valentinei	5	MS	Forrest	Pascagoula	COx1: KY658979	MMNS 19450	valentinei_BlkCrk_1	This study	*c*	*c*
D. valentinei	6	MS	Neshoba	Pearl	COx1: KY658984	JYL474	valentinei_Pearl_BlkCrk_2	This study	32.9078	88.9915
D. valentinei	6	MS	Neshoba	Pearl	COx1: KY658984	JYL475	valentinei_Pearl_BlkCrk_2	This study	32.9078	88.9915
D. valentinei	6	MS	Neshoba	Pearl	COx1: KY658982	MMNS 19453	valentinei_Pearl_2	This study	32.9078	88.9915

TABLE 1. (Continued)

Species	Locality #	State	County/Parish	Drainage	Accession#	Specimen#	Mitochondrial Haplotype	Source	Lat.	Long.
D. valentinei	6	MS	Neshoba	Pearl	COx1: KY658982	MMNS 19454	valentinei_Pearl_2	This study	32.9078	88.9915
D. valentinei	7	MS	Wayne	Pascagoula	COx1: KY658980	MMNS 19446	valentinei_Chick_1	This study	31.616	88.8821
D. valentinei	8	MS	Harrison	Biloxi	COx1: KY658984	MMNS 19455	valentinei_Pearl_BlkCrk_2	This study	c	c
D. valentinei	8	MS	Harrison	Biloxi	COx1: KY658984	MMNS 19457	valentinei_Pearl_BlkCrk_2	This study	c	c
D. valentinei	8	MS	Harrison	Biloxi	COx1: KY658984	MMNS 19458	valentinei_Pearl_BlkCrk_2	This study	c	c
D. valentinei	0 (Topotype)	LA	St. Tammany	Pearl	CytB: KY659029	DBM3242/A21484	valentinei_topotype	This study	30.5681	89.9278
D. welteri	-	KY	Harlan	Tennessee	EU311675	DAB326	welteri_EU311675	Beamer & Lamb (2008)	-	-
D. wrighti	-	NC	Mitchell	a	AY728225	MVZ 222618	wrighti_AY728225	Mueller et al. (2004)	-	-
Eurycea bislineata	-	PA	Westmoreland	a	AY728217	MVZ 225074	Eurycea_AY728217	Mueller et al. (2004)	-	-
Phaeognathus hubricthii	-	AL	Butler	a	AY728233	MVZ 173507	Phaeognathus_AY728233	Mueller et al. (2004)	-	-

[a] State, county, and or drainage data were not provided by source
[b] An accession and or specimen number were not provided by the source
[c] Coordinate data are withheld due to the sensitivity of the site but are available from the MMNS
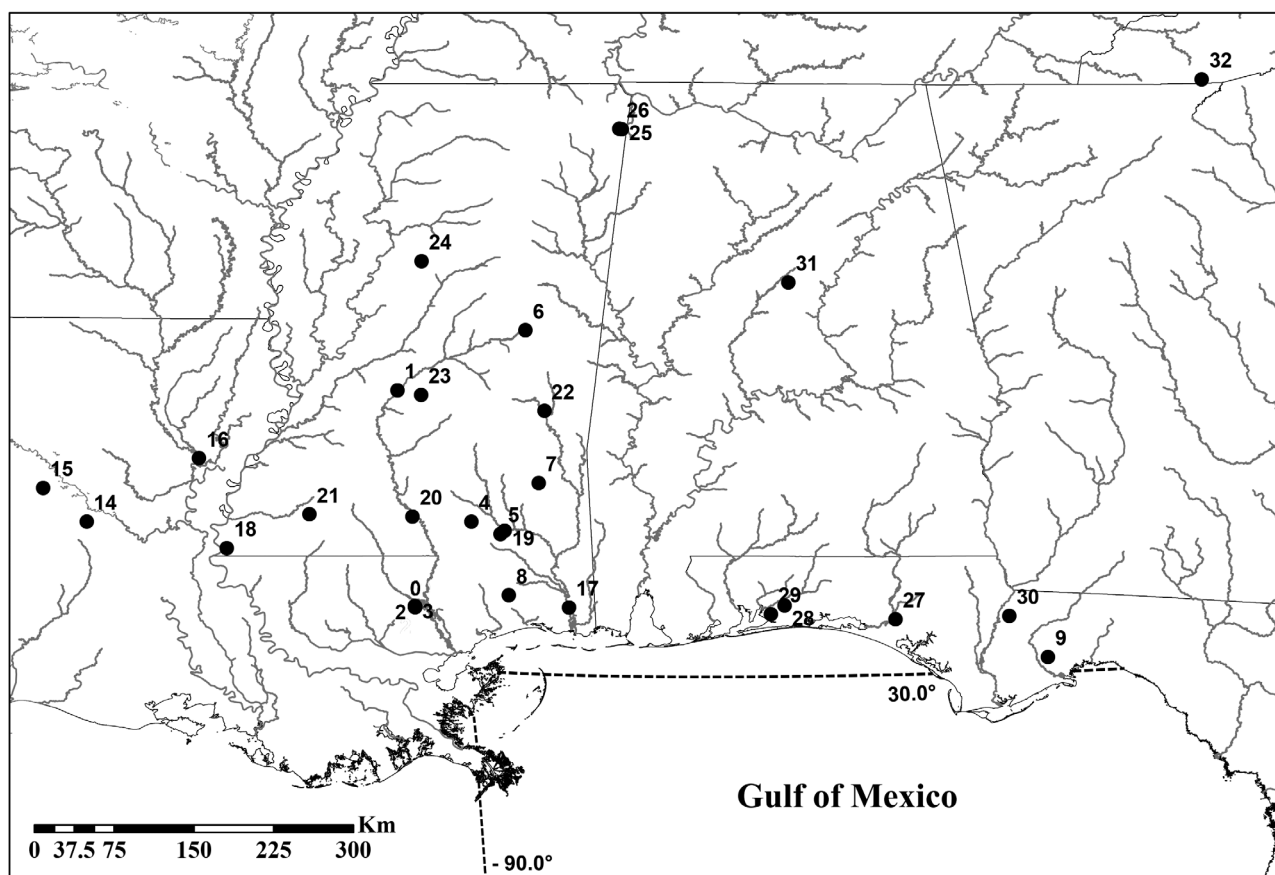
FIGURE 1. Collection localities for novel sequences from multiple species of *Desmognathus*. Site numbers correspond with locality data in Table 1.

We used TCS 1.21 (Clement *et al.* 2000) to identify unique mitochondrial haplotypes among the individuals we sequenced, and we analyzed these data alongside previously published sequences from other populations and species of *Desmognathus* (Table 1). We used MrBayes 3.2.5 (Ronquist *et al.* 2012) to construct a phylogeny from concatenated COx I and CytB sequences. We partitioned the dataset by gene in Mesquite (Maddison and Maddison 2015) and used jModelTest and Akaike's Information Criterion (Darriba *et al.* 2012) to determine the appropriate evolutionary model for each mitochondrial gene (COx I and CytB = HKY + I + G). We ran two, independent Monte Carlo Markov Chain analyses using four simultaneous chains 5×10^6 generations in length and a sampling frequency of every 100 generations. We assessed convergence using both split standard deviation (< 0.01) and minimum ESS values (> 1,000), and by plotting log linear data from the Markov Chains. We discarded 21,100 samples as burn-in, leaving a total of 28,900 sampled trees for which we calculated posterior probability support values in MrBayes using the sump and sumt commands. We viewed and formatted the 50% Bayesian consensus phylogram in FigTree (Rambaut and Drummond 2009) and Inkscape, an open-source vector graphics editor. We determined the number of parsimony informative sites for each mitochondrial gene and used COx I data to calculate net evolutionary divergence estimates (i.e., average uncorrected p-distances accounting for intragroup variation) among pertinent mitochondrial clades with greater than 0.95 posterior probabilities (hereafter pp) in MEGA 6.0 (Tamura *et al.* 2013).

Ongoing or historic hybridization is not uncommon among species of plethodontids and caution should be used when relying solely on mitochondrial haplotype data to describe different forms of *Desmognathus* (Tilley *et al.* 2013, Tilley 2016). Mitochondrial and species trees can be discordant where hybridization has occurred, and, as a consequence, individuals may incorrectly be assigned to an evolutionary lineage if researchers only utilize data from this genome. We sequenced RAG-1 for two individuals of *D. auriculatus* from Florida, three of the proposed *D.* **sp. nov.** from populations in Mississippi and Louisiana, and three presumptive *D. conanti* from Mississippi and Florida (Table 1) to determine whether individuals belonging to these mitochondrial lineages were also divergent in

their nuclear DNA. We obtained sequence data for both the forward and reverse strands for each individual of *D. auriculatus* and **D. sp. nov.,** but we only sequenced the forward strand for individuals of presumptive *D. conanti.* Seven of the eight individuals sequenced were heterozygous at one or more positions. We used the program DnaSP 5.10.1 (Librado and Rozas 1995) and the PHASE method (Stephens *et al.* 2001, Stephens and Donnelly 2003) to identify RAG-1 haplotypes and to determine whether there were shared alleles among lineages. We used these phased nuclear sequences in MEGA 6.0 to calculate net evolutionary divergence estimates among representatives of four mitochondrial lineages, including *D. auriculatus,* the proposed **D. sp. nov.**, presumptive *D. conanti* from the Gulf Coastal Plain, and, for comparative purposes, *D. quadramaculatus* (Table 1*).* We used RAG-1 haplotypes from *D. auriculatus,* **D. sp. nov.,** and presumptive *D. conanti* in TCS 1.21 to complete a statistical parsimony network analysis using a 95% parsimony criterion. Networks identified by TCS 1.21 were recreated in Inkscape.

Morphology. Measurements were taken with dial calipers to the nearest 0.1 mm, or in the case of a few individuals measured in the field, with a ruler and to the nearest 1 mm. Snout-vent length (SVL) was measured from the tip of the snout to the posterior margin of the vent. Osteological comparisons were made from skulls of the new species described herein, **D. sp. nov**. (altogether 5 adults from Mississippi and Louisiana) and *D. auriculatus* (altogether 6 adults from Georgia and Florida) that were dissected and then hand-cleaned using jeweler's forceps (see Means 1974), as well as from high resolution Computed Tomography scans (voxel resolution = 10.7–14.7μm). These scans were produced on a GE Phoenix Vtomex M CT scanner at the University of Florida's Nanoscale Research Facility, reconstructed using Phoenix's proprietary Datos software, and analyzed in VGStudio Max 2.2. (Volume Graphics, Heidelberg, Germany). The resulting shape files and tiff-stacks of the specimens presented here are available at morphosource.org (direct link Bit.ly/UFHerpMorph). Measurements were made from tomograms with the measuring tool in Adobe Photoshop, Version 10.0.

The premaxillary fontanelle of metamorphosed specimens was measured from dorsal view CT-scan images along a medial line from the anterior tip of the fused premaxillary bones to the posterior margin of the fontanelle. This measurement was divided by the length of the ramus of the rightmost premaxillary bone from its posterior tip to the anterior tip of the fused premaxillary bones, also taken from the same CT-scanned dorsal views of the skull. The resulting dividend was the proportion of the length of the ramus of the right premaxillary bone that constituted the length of the premaxillary fontanelle. Whether the two rami of the premaxillary bones were fused or not was also scored from the CT scans.

Meristic data from larvae resulting from courtship trials with Mississippi populations, as well as for a limited number of larvae caught in the field, were collected by JYL from photos taken with an Olympus E-620 digital SLR camera and analyzed with tpsDig2 software (Rohlf 2006; see section on Reproduction). JYL also weighed 6 randomly chosen hatchlings, and later 10 larvae, from a lab produced clutch on an OHAUS Explorer ® ver. 1.10 scale. Photos and masses were collected within 24 hours of hatching, as well as again ca. once per month until gill and tail-fin absorption began.

Museum Collection Abbreviations: **MCZ** = Museum of Comparative Zoology, Harvard University, Cambridge, USA; **MMNS** = Mississippi Museum of Natural Science, Jackson, USA; **USNM** = United States National Museum/Smithsonian Institution, Washington, D. C., USA; **UF** = Florida Musuem of Natural History, University of Florida, Gainesville, USA.

TABLE 2. Primers used for amplification and sequencing. Asterisks indicate those primers used only in sequencing.

Gene	Primer Name	Primer Sequence (5' to 3')	Reference
COx I	Beamer_Lamb_F	CGGCCACTTTACCYRTGATAATYACTCG	Beamer and Lamb (2008)
	Beamer_Lamb_R	GTATTAAGATTTCGGTCTGTTAGAAGTAT	Beamer and Lamb (2008)
CytB	MVZ 15	GAACTAATGGCCCACACWWTACGNAA	Moritz *et al.* (1992)
	cyt b2	AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA	Moritz *et al.* (1992)
	DesmogCytBR	TGGTTTACAAGACCAATRTTTTTC	This study
RAG-1	DESMOG_Rag1_F	CGGCAGATATTCCAGCCTTTAC	Bonett *et al.* (2014)
	DESMOG_RAG1_r	CGATGGAGCCATCTCGCTCTATGA	Bonett *et al.* (2014)
	*Desmog_Rag1F_BK	AATGGACTGTCTGGTCTGGTC	This study
	*Desmog_Rag1R_BK	TGGTGATCTTGCCTTCATACC	This study

Results

Molecular analyses. We generated 47 COx I (531 bp in length), 30 CytB (367 - 1082 bp), and 8 RAG-1 (1077 - 1090 bp) sequences for this study. In the dataset used in the Bayesian analysis there are a total of 200 and 400 parsimony informative sites for COx I and CytB, respectively. Our phylogeny recovers Beamer and Lamb's (2008) four reciprocally monophyletic groups of *D.* cf. *auriculatus* (pp ≥ 0.95), which they labeled C2–C5, and which represent lineages of unclarified taxonomic status. It also recovers a clade (pp ≥ 0.95) containing sequences from Liberty Co., Georgia, which is very near the topotype locality for *D. auriculatus* sensu stricto, and which Beamer and Lamb (2008) labeled C1 (Fig. 2). Like Beamer and Lamb (2008), we propose that populations within this clade are the only ones to which the epithet *auriculatus* correctly applies.

Individuals from Louisiana and Mississippi that we identify as ***D. sp. nov.*** constitute a new, genetically disparate monophyletic group (pp ≥ 0.95) more closely related to *D. conanti* (Rossman), *D. brimleyorum* (Stejneger)*,* and *D. santeetlah* (Tilley) (pp = 0.94) than to *D. auriculatus* or any clade of *D.* cf. *auriculatus* (Fig. 2). However, given the polytomy, we are unable to identify which clade is sister to ***D. sp. nov.*** In Kozak *et al.* (2005) there are several sequences identified as *D. auriculatus* from localities in central Mississippi (their Site 69) which nest within a clade containing presumptive *D. conanti*. We suggest that these individuals from Mississippi have been misidentified and are neither *D. auriculatus* nor ***D. sp. nov.*,** but instead belong to a clade currently labeled with the *conanti* epithet. A fragment of COx I is available for one of the Kozak *et al.* individuals (GenBank AY612415), and the portion of this sequence which aligns with our COx I sequences (99 bp) is identical to our "conanti_Pearl_3_24" haplotype from the Pearl Drainage in neighboring Scott Co., Mississippi (Table 1, Fig. 2). Although the overlapping sequence from Kozak *et al.* is short, it encompasses a variable portion of COx I and there are eight nucleotide differences between it and sequences from those individuals that we identify as ***D. sp. nov.*** We do not consider it likely that the Kozak *et al.* salamanders are the result of hybridization between ***D. sp. nov.*** and sympatric *D. conanti.* JYL staged captive courtship trials between presumptive *D. conanti* and ***D. sp. nov.*** from populations in the Pearl and Pascagoula Rivers in Mississippi and although many homospecific pairings resulted in insemination, no heterospecific pairings between ***D. sp. nov.*** and presumptive *D. conanti* progressed past initial pheromone transfer behaviors. This suggests that these taxa are sexually isolated (Lamb in press).

Individuals that were originally identified by both ourselves and Beamer and Lamb (2008) with the *conanti* epithet occur across multiple clades within a moderately supported group (pp = 0.90) that also contains *D. santeetlah* (Fig. 2). It may be the case that there is cryptic diversity among populations currently subsumed by *D. conanti* in the Gulf Coastal Plain (Kozak *et al.* 2005, Lamb 2016), but a detailed systematic and phylogeographic analysis of *D. conanti* (sensu lato) is beyond the scope of this work. Morphological and molecular analyses involving the *D. conanti* species complex are ongoing. The net evolutionary divergence estimate for COx I between ***D. sp. nov.*** and presumptive *D. conanti* from drainages East of the Mississippi River in which these taxa are sympatric is high (8.55% between ***D. sp. nov.*** and the clade of presumptive *D. conanti* marked with a star in Fig. 2), as are those estimates between ***D. sp. nov.*** and other clades (Table 3).

The genetic distinctiveness of ***D. sp. nov.*** as demonstrated from our analyses of mitochondrial sequence data is further bolstered by our analysis of the nuclear gene RAG-1. We trimmed RAG-1 sequences to a total length of 1077 bp. DnaSP identified 12 haplotypes among the eight salamanders sequenced for this study. None of these haplotypes were shared between individuals belonging to different mitochondrial lineages. The 95% statistical parsimony network analysis drew two networks separated by >14 mutational steps, one containing only ***D. sp. nov.*,** and the other containing presumptive *D. conanti* and *D. auriculatus* (Fig. 3). The estimated net evolutionary divergence (± standard error) between *D. auriculatus* and ***D. sp. nov.*** is 1.15% ± 0.30, and that between ***D. sp. nov.*** and sympatric *D. conanti* is 1.08% ± 0.29. For comparison, the average of this value between published sequences for *D. quadramaculatus* (Table 1) and any of these three groups is 1.40% ± 0.15. Similarly high levels of differentiation have been observed when making interspecific comparisons of RAG-1 sequences among other plethodontids (Elmer *et al.* 2013), as well as among other vertebrates (Cortés-Ortiz *et al.* 2014).

The genetic evidence suggests that salamander populations from the Gulf Coastal Plain currently assigned to *D. auriculatus* belong to distinct lineages that are not each other's closest relatives. Deeply divergent mitochondrial clades among other *Desmognathus* within this region may also reflect hidden taxonomic diversity, but these lineages will require additional studies prior to taxonomic revision. However, we consider the distinctiveness of the two Gulf Coastal Plain lineages currently assigned to *D. auriculatus* to be substantial, and we begin drawing

taxonomic conclusions for *Desmognathus* in this region by describing **D. sp. nov.** as new species and by providing a precise redescription of *D. auriculatus*.
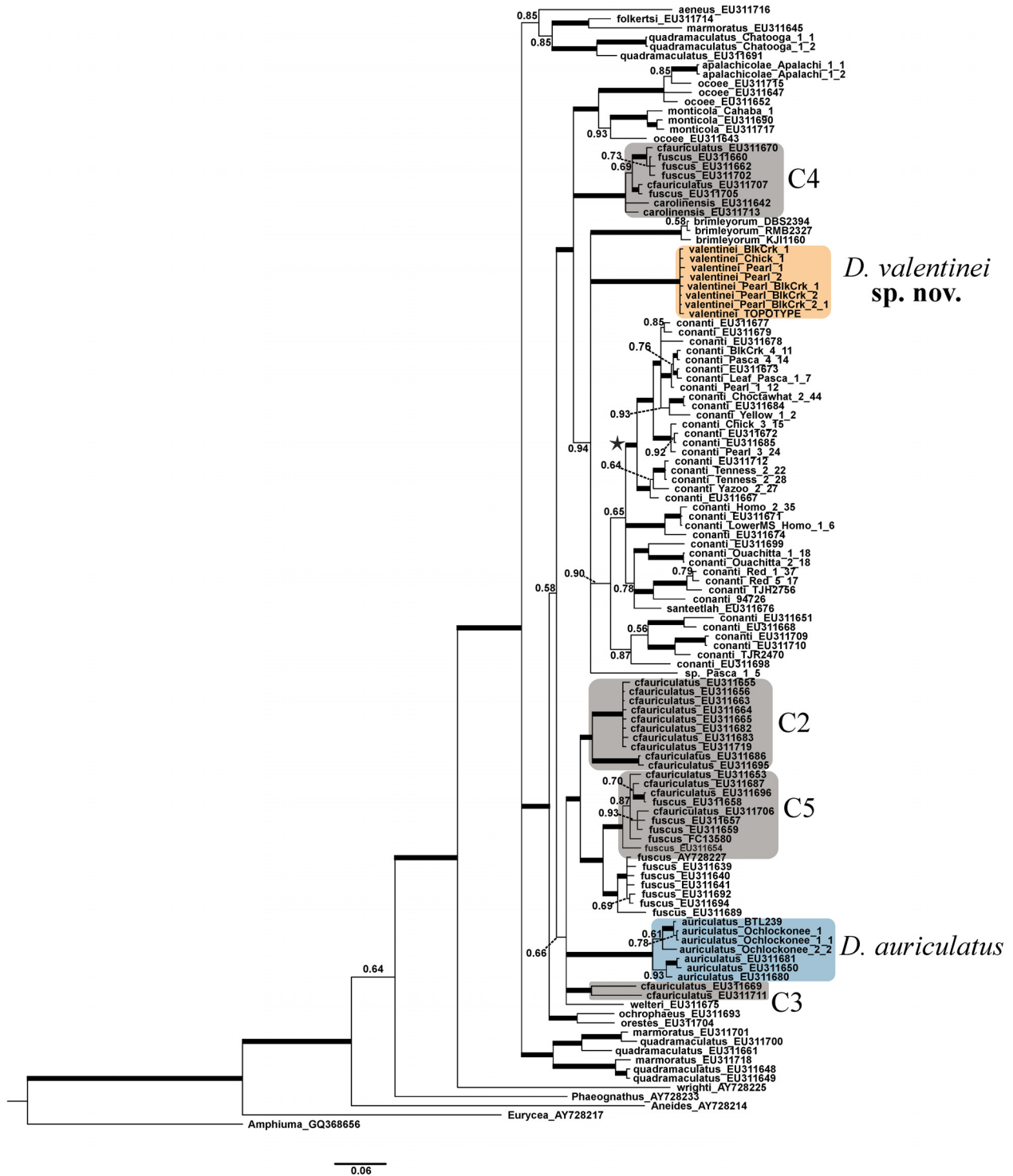

FIGURE 2. Bayesian 50% majority rule consensus phylogram of concatenated COx I and CytB sequences. Posterior probabilities are based on 28,900 post-burn-in trees which had an average marginal likelihood score of -15337.63. Thicker branches indicate probabilities ≥0.95. Probabilities <0.50 are not shown. ***Desmognathus* sp. nov.** is indicated in orange, *D. auriculatus* in blue, and clades C2–C5 of *D.* cf. *auriculatus* from Beamer & Lamb (2008) in grey. The larger clade containing *D. conanti* (sensu lato) and marked with a star was used when calculating net evolutionary divergence estimates (Table 3). Taxa labels correspond with Table 1.
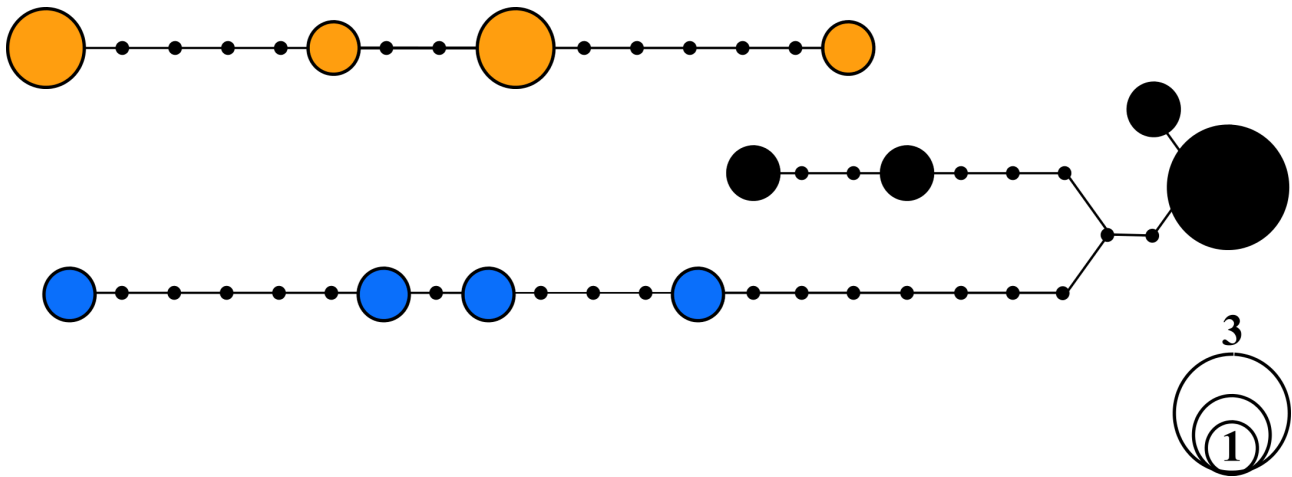
FIGURE 3. Nintey five percent statistical parsimony networks for RAG-1 haplotypes. Haplotypes belonging to **D. sp. nov.** are indicated in orange, those for *D. auriculatus* in blue, and those for presumptive *D. conanti* in black. No alleles were shared between lineages. Circle size represents the number of alleles of that particular haplotype (see legend in bottom right) and black dots represent the number of mutational steps between observed haplotypes. Networks are separated by >14 steps.

TABLE 3. Estimates of net evolutionary divergence based on COx I between mitochondrial clades of *Desmognathus* as calculated in MEGA 6.0. Net average p-distances between clades (as percentages) and standard error estimates are given below and above the diagonal, respectively. Redundant sequences were not removed prior to calculations. Those presumptive *D. conanti* included in these calculations occur within the clade marked by a star in the Bayesian analysis (Fig. 2). *Desmognathus wrighti* is provided for comparison and clade labels used in Beamer & Lamb (2008) are given in parentheses.

	auriculatus (C1)	cf. *auriculatus* (C2)	cf. *auriculatus* (C3)	cf. *auriculatus* (C4)
auriculatus (C1)		1.03	0.97	1.03
cf. *auriculatus* (C2)	7.61		0.74	1.04
cf. *auriculatus* (C3)	5.57	3.61		0.88
cf. *auriculatus* (C4)	7.95	7.06	4.83	
cf. *auriculatus* (C5)	8.24	5.08	4.36	7.96
sp. nov.	12.09	9.67	8.34	9.03
conanti	8.08	6.99	4.07	5.67
brimleyorum	9.35	9.11	5.46	7.7
wrighti	16.72	15.08	12.05	14.8

continued.

	cf. *auriculatus* (C5)	sp. nov.	*conanti*	*brimleyorum*	*wrighti*
auriculatus (C1)	1.11	1.32	1.1	1.18	1.59
cf. *auriculatus* (C2)	0.81	1.27	1.02	1.19	1.48
cf. *auriculatus* (C3)	0.82	1.2	0.78	0.93	1.37
cf. *auriculatus* (C4)	1.14	1.18	0.88	1.04	1.48
cf. *auriculatus* (C5)		1.41	1.06	1.24	1.46
sp. nov.	12.14		1.1	1.41	1.68
conanti	7.66	8.55		0.98	1.49
brimleyorum	10.26	12.28	6.71		1.59
wrighti	14.8	19.21	15.93	16.95	
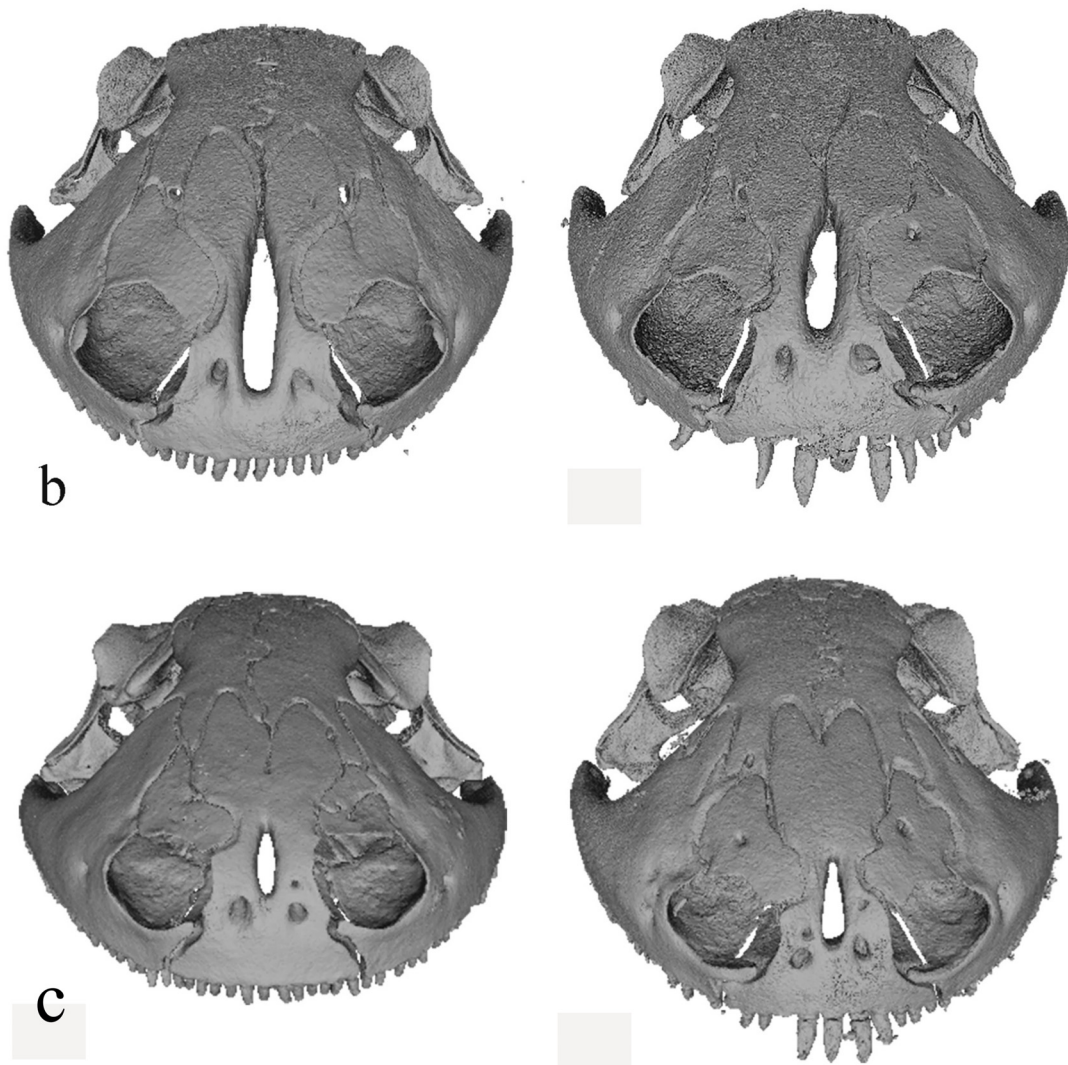
FIGURE 4. Cranial osteology of **a** = dorsal views of *D. valentinei* **sp. nov.** from Louisiana (left to right, female and male) compared with topotypical *D. auriculatus* (female, male) from Liberty Co., GA, and **b** = frontal views of female and male *D. valentinei* **sp. nov.** from type locality versus **c** = female and male *D. auriculatus* from near its type locality. Note that the premaxillary fontanelle of *D. valentinei* **sp. nov.** is proportionately much larger than in *D. auriculatus* and their rami are fused or partially fused. Scale in 4a = 1.0 mm.

Morphological comparisons. Paired premaxillary bones is the norm in vertebrates (Romer and Parsons 1986), but they are fused anteriorly in desmognathan salamanders, providing structural strength to the tip of the snout and enabling the head to be used more effectively as a wedge for burrowing (Means 1974). In *Desmognathus* each of the anteriorly fused premaxillaries has a backward projecting ramus that is rarely (most species) or sometimes fused posteriorly (*D. marmoratus*), but always has an elongate, non-fused fontanelle where the two rami first project backward. In most *Desmognathus* this fontanelle comprises 30-50% of the length of the rami. However, Means (1974) demonstrated that the premaxillary fontanelle was much reduced in topotypic *D. auriculatus* in comparison with Florida populations he called *D. fuscus* (Rafinesque), which subsequently have been referred to *D. apalachicolae* and *D.* cf. *conanti* (Means and Travis 2007). The reduced premaxillary fontanelle in *D. auriculatus* was thought to facilitate burrowing in viscous muck and peat habitats (Means 1974). The premaxillary fontanelle in adults of both sexes of **D. sp. nov.** (n = 5) was measured to be at least 40 percent of the length of the ramus of the right premaxillary bone, whereas in *D. auriculatus* (n = 6) the fontanelle ranged from 13–22 percent (Fig. 4a). The two rami of the anteriorly fused premaxillary bones had a clearly resolved suture between them in CT scans of the skulls of two males and two females of *D.* **sp. nov.** (Fig. 4b), but in three males and two females of *D. auriculatus* no suture was visible and the rami were fused posterior to the small fontanelle (Fig. 4c).
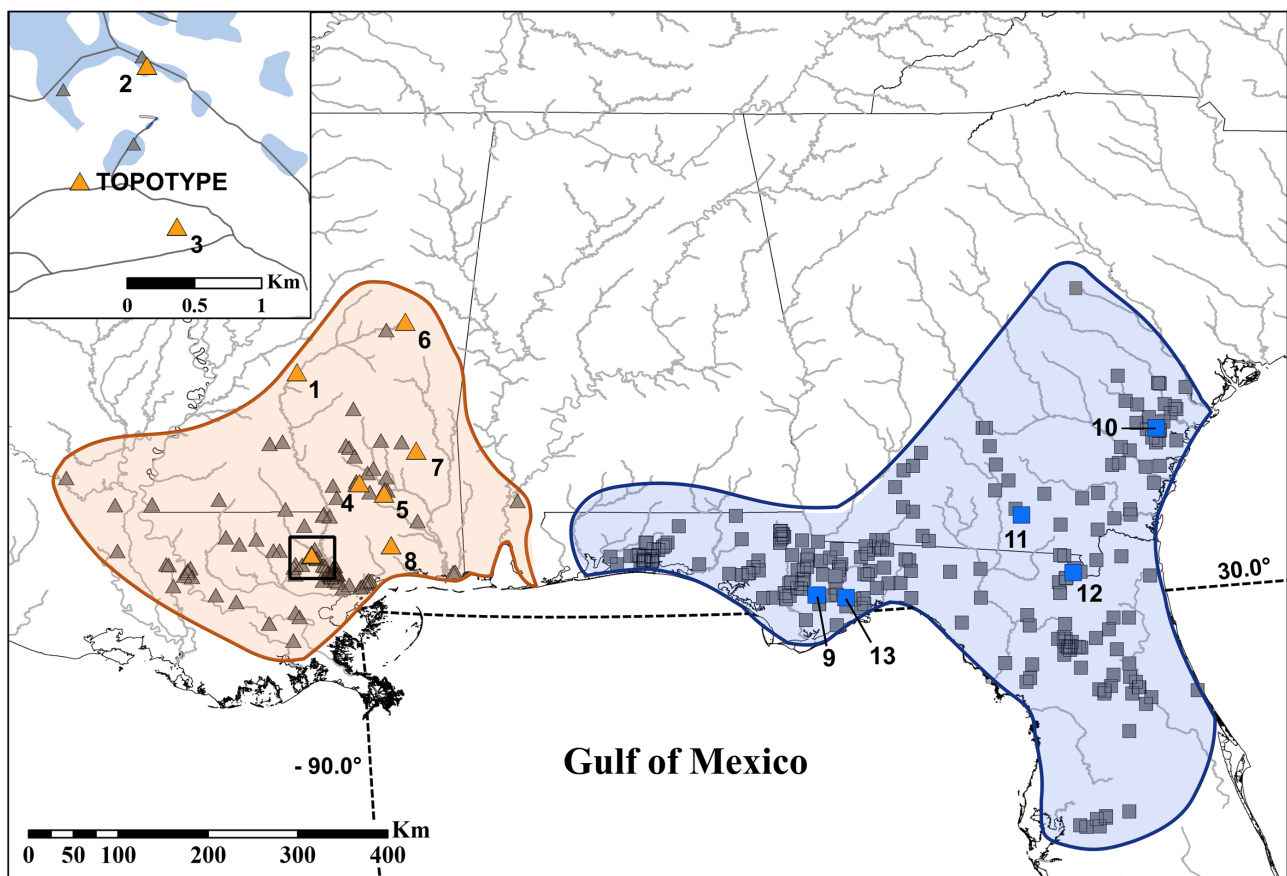
FIGURE 5. Range map for **D. valentinei sp. nov.** (orange) and *D. auriculatus* (blue) based on museum specimens and genetic analyses. Triangles represent **D. valentinei sp. nov.,** and squares *D. auriculatus*. Color corresponds with data used to assign each locality to either species (gray = museum specimen[s] only; orange and blue = sequence data from up to three genes). Locality numbers correspond with those in Table 1 and Figure 1.

These morphological differences together with the mitochondrial and nuclear sequences distinguish **D. sp. nov.** from topotypic *D. auriculatus*. Taxonomic revision is thus required to reflect this cryptic evolutionary diversity. Herein we formally describe **D. sp. nov.** whose morphology, ecology, life history, and distribution we have studied intensively. We also present a redescription of the now restricted taxon *Desmognathus auriculatus*. A restriction of the *auriculatus* epithet was also suggested by Beamer and Lamb (2008) based on their COx I dataset, however formal naming of their other lineages (i.e., *D.* cf. *auriculatus* [Fig. 2], Beamer and Lamb Clades 2–5)

must await further studies. Our genetic data and examination of vouchered specimens reveals that populations that were previously identified as *D. auriculatus* from the Mobile River at the head of the Mobile Bay west to the eastern side of the Lower Mississippi River Valley form a well-supported, reciprocally monophyletic group that is disparate from a clade containing topotypic *D. auriculatus*. These taxa currently appear to be allopatric, but see below (Fig. 5). Further, this Gulf Coastal Plain clade of ***D. sp. nov.*** is also monophyletic with respect to other sympatric populations of *Desmognathus* (Fig. 2; Lamb 2016). Based on the concordant genetic differentiation in mitochondrial and nuclear genes, and differences in cranial osteology in a limited number of specimens, we now take pleasure in naming this clade.

New species description

***Desmognathus valentinei* sp. nov.**
Figs. 4–12.

Suggested common name: Valentine's Southern Dusky Salamander
Holotype. MCZ A-143850 (field collection DBM-3242), an adult male with two black lobes on each testis (Fig. 6a, b top), 61.3 mm SVL, collected from Talisheek Bay swamp, ~20 m elevation, east of Money Hill Road, 30.568056° N, 89.927778° W, in St. Tammany Parish, Louisiana, by D. Bruce Means and Jeff Boundy, 20 April 2005.
Paratopotypes. MCZ A-143845, -46, -47, -48, -49, -51, -52, -56, -57, -58: same collection data as the holotype.
Paratypes. MMNS-19445 to 19447, JYL field tags 249, 160, and 115, collected from Thompson Creek upstream of its crossing with Strengthford Cooley Rd, 31.61597° N, 88.88214° W, Wayne Co., Mississippi, by Jennifer Y. Lamb. MMNS-19448 to 19449, JYL field tags 169 to 170, collected from bottomland seep-fed depressions along Perkins Creek north of the intersection between Old Hwy 24 and Cole Rd., 31.2898° N, 89.45022° W, Lamar Co., Mississippi, by Jennifer Y. Lamb. MMNS-19450 to 19452, JYL field tags 256, 260, and 372, collected from Camp Shelby, Forrest Co., Mississippi, by Jennifer Y. Lamb and James R. Lee. MMNS-13862 to 13863, collected from below the Ross Barnett Reservoir Dam, 32.39311° N, 90.06076° W, Madison Co., Mississippi by J. Scott Peyton. MMNS-19453 to 19454, JYL field tags 472 to 473, collected from a creek swamp flowing in to the Nanih Waiya Creek, 32.90777° N, 88.99146° W, Neshoba Co., Mississippi, by Jennifer Y. Lamb. UF-170022 to -170030, DBM-1688, collected from Mississippi River swamp, 29.894806°N, 90.377528°W, St. Charles Parish, Louisiana, by D. Bruce Means and James F. Berry on 8 April 1972.
Etymology. Named for Barry D. Valentine, who originally recognized the distinctiveness of this new species in Mississippi from shaded, small-order stream populations of other Coastal Plain species of *Desmognathus*, and color differences with topotypic *D. auriculatus* (Valentine 1963).
Diagnosis. *Desmognathus valentinei* is a medium-sized (adult males x̄ = 56.49 ± 5.59 mm SVL, N = 33; adult females x̄ = 53.28 ± 5.16 mm SVL, N = 30) member of the genus *Desmognathus* with a distinctively blade-shaped tail. It can be distinguished from other species of *Desmognathus* with which it is sympatric by its 1) larger body size (*D. conanti* [sensu lato] from populations in the Homochitto, Pascagoula, and Pearl River drainages: adult males, x̄ = 48.63 ± 4.49 mm SVL, N = 23; adult females x̄ = 48.26 ± 9.19 mm SVL, N = 30); 2) nondescript dorsal markings in adults, which are unlike the crisply defined pairs of colored blotches typical of many *Desmognathus*; 3) often a bright orange to reddish-brown dorsal coloration on the anterior half of the tail; 4) bladelike, laterally compressed tail rather than a round to trigonal tail that continuously narrows to a fine-pointed tip; 5) ventral coloration, which is a mottling of dark and tan with very few iridophores; and 6) living in low-gradient, slow-water habitats having soupy muck rather than first- and second-order (Strahler 1964), usually sandy-bottomed streams. Each of these characteristics distinguishes *D. valentinei* from sympatric *D. conanti* (Valentine 1963) and from allopatric *D. brimleyorum* (Means 1974). Sexually mature *D. brimleyorum* can reach very large sizes (> 70 mm SVL) and the two species also differ in their cranial osteology (e.g., *D. brimleyorum* has distinctive fungiform teeth, whereas *D. valentinei* does not) (Means 1974).
Description of holotype. An adult male (MCZ A-143850) with densely black, convoluted vasa deferens and two enlarged, black lobes on each testis. Measurements in millimeters after ten years in preservation, taken as in

Tilley (1981) and Anderson and Tilley (2003): SVL = 61.3; head length = 3.51; head width at jaw musculature = 16.1, and at jaw angle = 9.35; trunk width at axilla = 8.74; trunk width at pelvis = 7.57; tail length = 45.2 (extreme tail tip bent 90˚ during preservation but the posterior one-third may possibly be regenerated); tail width at posterior margin of the vent = 6.88; tail height at base = 7.73; tail width and height at fifth caudal fold = 6.25, 6.26; a U-shaped depression along the dorsal midline of the proximal portion of the tail becomes flat by the fourth caudal fold and then distally a fleshy fin rises prominently towards the tail tip, culminating in a distinct ridge on the distal 4/5 of the tail; left forelimb length = 8.10; toe lengths on left manus (medial to lateral) = 0.09, 1.30, 1.90, 1.10; left hind limb length = 3.27; toe lengths on left pes (medial to lateral) = 1.00, 2.21, 2.36, 2.81, 2.05. The holotype has 14 costal grooves (12 + axilla + groin) on the left side and six costal folds between the adpressed limbs. A sinuate groove strongly indents the skin running horizontally from the back of the eye above the light cheek patch to the gular fold; below the groove the cheek is lighter gray with six or seven tiny, faint, light gray round spots. Jaw teeth are of the piercing type (Means 1974, Caldwell and Trauth 1979) and the commissure is straight to very slightly downcurved anteriorly. The gular fold is prominently indented across the undersurface of the neck. The mental gland at the tip of the undersurface of the lower jaw is 2.3 mm long (axially) by 3.1 mm wide (laterally).

FIGURE 6. a = live holotype (MCZ A-143850) adult male ***Desmognathus valentinei*** **sp. nov.** from Talisheek Bay, St. Tammany Parish, LA; **b** = holotype above and gravid female (unavailable as a paratype) below. Notice the longer head and snout and larger "jowls" of the male.
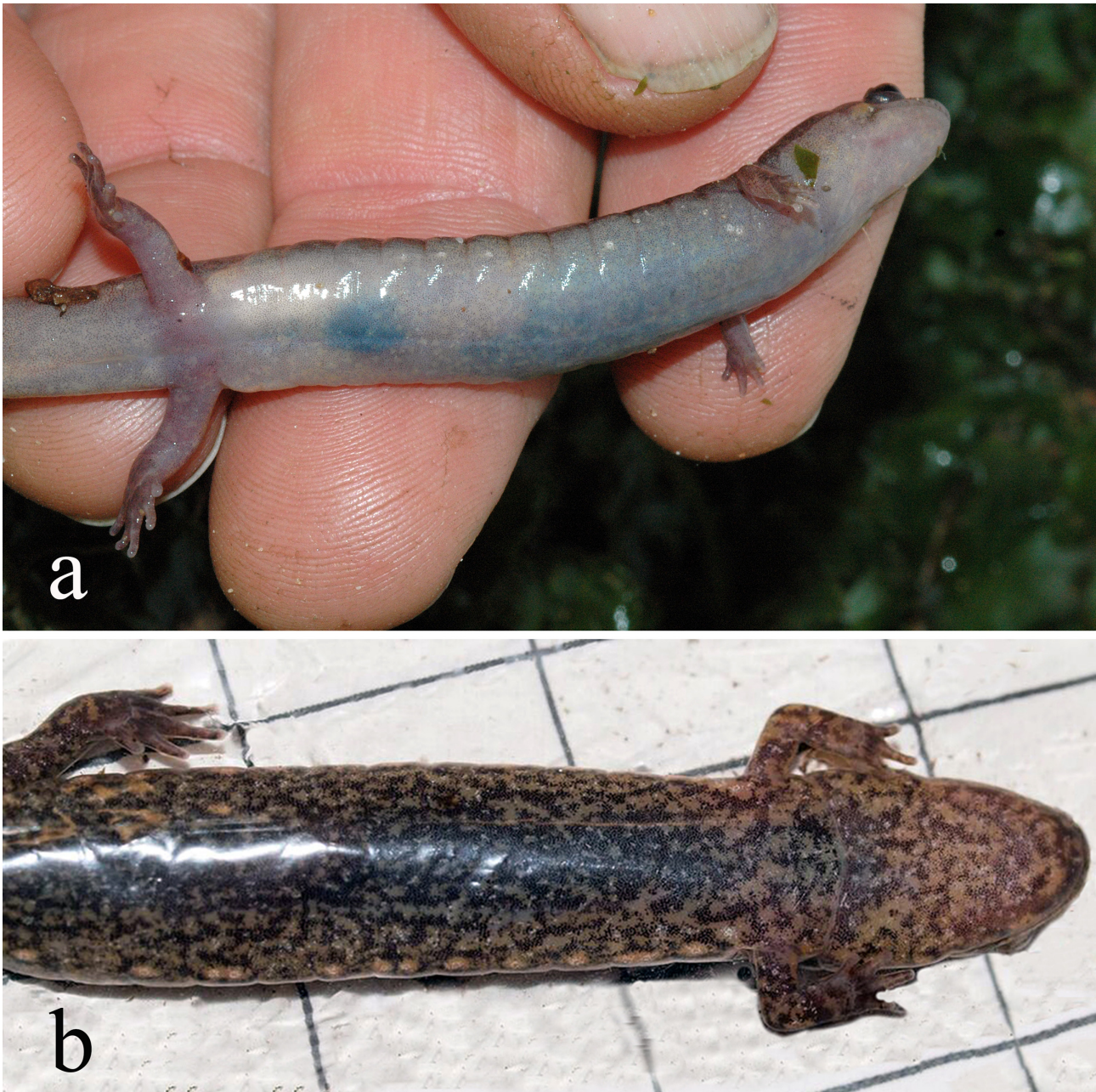
FIGURE 7. a = belly of same gravid female as in Fig. 6b from tannish loess muck; **b** = female paratype (MMNS 19447) from Strengthford, Wayne Co., MS showing melanophores of the belly pattern in stellate condition.

Coloration of live holotype. The color pattern of the living holotype was photographed 18 hours after collection using a flash on a digital camera at F 35 and speed of 1/60th sec when the specimen had lightened up slightly via metachrosis (Fig. 6a, b top). The dorsolateral surfaces of its head and body to the hind limbs is medium gray with a dense sprinkling of punctate black melanophores, some of which are organized into a faint network of very thin, short dark lines on top of the head and neck. A very faint, thin black line runs down the middle of the back and some of the melanophores are aggregated into a few (< 10) small dark blotches less than the width of a costal fold. The dorsolateral darker gray color gives way ventrolaterally to lighter gray with many fewer punctate melanophores, but with a row of prominent pinkish-orange or whitish-tan "portholes," one per costal fold. The classic desmognathine cheek patch of light color angles down and backward from the posterior of each eye to the corner of the commissure. It is the same pinkish-orange color of the portholes and of a prominent, narrow dorsal stripe down the first half of the tail. The sides of the tail are gray like the dorsum, but has a broken line of fainter dorsolateral portholes and a line of stronger appearing ventrolateral portholes. The undersurfaces of the chin, belly,

and tail are densely peppered with melanophores in punctate condition but having faint, pigmentless blotches about the diameter of the portholes or smaller, but regularly distributed throughout. The top of the arms and legs are colored as on the dorsum. Toe-tip friction pads as described by Caldwell and Trauth (1979) are not present. The belly color of the holotype was not photographed but was similar to that of a female collected at the same time and place (Fig. 7a). It was basically white with a faint veneer of punctate melanophores. A female collected from Strenghtford, Wayne Co., MS (MMNS 19447), had the same pattern but the melanophores were in stellate condition (Fig. 7b) rendering a much darker specimen.

Coloration of the preserved holotype. After 10 years in 70% ethanol the color pattern of the holotype was not strongly different from when alive (Fig. 8 a,b,c). Dorsally, it maintained the color when alive except that the dark pigment is slightly lighter overall and the tannish orange color of the dorsum of the tail is weaker. When killed, the holotype was dissected to remove some internal organs for electrophoresis, but the testes remain. The ventral surfaces of the chin, neck, belly, and tail are uniformly lighter than the dorsum but an overwash of melanophores is still visible and they also faintly pepper the tissue surrounding the interior of the coelomic cavity (Fig. 8b).

FIGURE 8. Lateral, dorsal, and ventral views of the holotype (MCZ A-143850) of *Desmognathus valentinei* **sp. nov.** after 10 years in 70% ethanol.

Eggs, hatchlings, and larvae. Eggs are typical of many *Desmognathus* in that they are deposited in small, compact clusters and cling to one another by extensions of the outer envelope (Fig. 9).

Larvae collected in the field and those obtained from clutches oviposited in captivity were similar in appearance (Fig. 10a,b,c). The background coloration is a dark brown or tan and is overlain by a mottling or streaking of black melanophores. Three rows of very small spots marking the presence of the larval lateral line organs (Means 1974) are visible on hatchling (Fig. 10a) and larval (Fig. 10b,c) *D. valentinei*. The first row occurs dorsolaterally on the body and continues onto the upper half of the tail musculature. In hatchlings, these spots are typically a lighter tan, though they are sometimes marked with white, and they are distally edged by a dense collection of melanophores. This first row of spots may either be paired opposite one another (usually 6–8 pairs), or

may alternate, and the distance between each spot is greater than the diameter of each spot. The second row of spots begins on the body in close proximity with the first row, but moves onto the tail musculature at about ½ the musculature's height (Fig. 10c). The third and final row can be seen ventrolaterally between the limbs and does not continue onto the tail (Fig. 10c). The shallow, dorsal tail fin originates posterior to the base of tail at ca. 1/3 to 1/2 the length of the tail, and it is mottled with melanophores (Fig. 10c). The typical, light or brightly colored desmognathan line from the posterior corner of the eye to the jaw is not strongly demarcated in hatchling or larval *D. valentinei*. There is a distinct, darkly pigmented, horizontal line across the pupil of the eye that can continue on to the rostrum towards but not past the nares. The hatchlings produced from the clutch that were oviposited in captivity (Lamb in press) averaged 12.11 ± 0.35 mm SVL (range 11.36–12.73 mm; N = 31) and 0.0597 ± 0.0022 g in mass (range 0.0562–0.0626 g; N = 6).

FIGURE 9. Female **D. valentinei** **sp. nov.** (MMNS 19446) from Strengthford, Wayne Co., MS with clutch of 31 eggs oviposited on October 2014 in captivity as part of a separate courtship study (Lamb in press).

As larvae of *D. valentinei* develop, they exhibit a more distinct pattern that retains some of the characteristics observed in hatchlings. Larval *D. valentinei* (Fig. 10b,c) do not develop the strongly demarcated, wide diameter dorsal spots that are often apparent in sympatric *Desmognathus*. Instead, they retain the subtle, first row of small-diameter spots or blotches, which can be an orange to red-brown color. The distal edges of these spots remain lined with dark melanophores, and these borders can merge to outline a dorsal orange to red-brown band with narrow out-pockets of white or lighter pigment (i.e., narrow scallops). This dorsal band sometimes contains scattered, dark flecks. On the tails of larval *D. valentinei*, the edges of the first row of spots may not completely merge but can sometimes form an irregular light area with opposing scallops. A strongly angular, zig-zag pattern, which is often present in larval, sympatric *D. conanti* (sensu lato), is not apparent at the base of the tail in larval *D. valentinei*. Older larvae and individuals undergoing metamorphosis are more darkly streaked or mottled on their sides. This pigment accentuates the second and third rows of small spots on the body and tail, which can be brilliantly white, particularly in recent metamorphs and small juveniles (Fig. 10d,e). The previously described pattern, relatively larger size at metamorphosis, as well as gill structure set larval *D. valentinei* apart from the larvae of sympatric

species of *Desmognathus*. Larval *D. valentinei* have uncharacteristically long rami for a desmognathan and they are mottled with dark pigment. This trait is apparent at hatching, as well as throughout the larval period. Valentine (1963) described the luxuriant external gills of 38 larvae from southern Mississippi as "First gill (anteroventral) five to seven fimbrae, usually six; second gill eight to sixteen fimbriae, twelve occurring most commonly; third gill (posterodorsal) eleven to seventeen fimbriae, almost two-thirds of the specimens having from thirteen to fifteen."

Recent metamorphs exhibit a light or white, irregularly edged line from the corner of the eye to the jaw, and they are ventrally mottled with melanophores and tan pigments. *Desmognathus valentinei* raised through metamorphosis in captivity (Fig. 10d) were similar in pattern and in coloring to small juveniles found in the field at Strengthford, Wayne Co., MS (Fig. 10e). At the onset of metamorphosis, individuals of *D. valentinei* from the laboratory hatched clutch averaged 20.21 ± 0.88 mm SVL (range 18.92–22.06 mm, N = 30) and 0.2482 ± 0.0422 g in mass (range 0.1857–0.3226 g, N = 10). Sizes at metamorphosis for individuals from this clutch produced in captivity may be smaller than what is typical for recent metamorphs in nature. The smallest metamorphosing individuals encountered by JYL in the field (i.e., still had visible remnants of gill stubs) were from Nanih Waiya Creek, Neshoba Co., MS, captured in late July 2015, and measured 26 and 29 mm SVL. Most of the smallest, fully metamorphosed individuals (i.e., no gill remnants) observed by JYL were captured in June and July in 2014 and 2015. These included four juveniles from Ross Barnett Reservoir, Madison Co., MS that were 23–32 mm SVL, two from Strengthford that measured 25 and 32 mm SVL, seven individuals from Nanih Waiya Creek that were 29–35 mm SVL, and a single individual from Perkins Creek, Lamar Co., MS that measured 32 mm SVL. Populations of *D. valentinei* may have variable ovipositional or larval periods, as small, metamorphosed individuals were also encountered at Camp Shelby, Forrest Co., MS (33–35 mm SVL; N = 3) and Perkins Creek (34 mm SVL; N = 1) in October 2014. The herpetology collection at Southeastern Louisiana University contains 10 individuals of *D. valentinei* from St. Tammany Parish, LA, all with remnant gill stubs (SLU #06579). These individuals averaged 34 ± 2 mm SVL (range 31–39 mm).

Adult body size. As with most desmognathan salamanders, the tail is usually partially regenerating. Total length is therefore not as conservative a character as is snout-vent length (SVL). From our sample, which includes individuals that were vouchered as well as individuals that were released after capture, the average SVL of 30 adult females was about 6 % shorter than the average SVL of 33 males. The largest male and female, both from the Camp Shelby Site, Forrest Co., MS, measured 72 and 62 mm, respectively. The smallest male and female in our sample each measured 42 mm SVL.

Variation. Valentine (1963) remarked that specimens from west of the Pearl River were lighter in color than those in southeastern Mississippi. We believe this is at least in part the result of color-matching with the muddy, glacial loess of the swampy habitats west of the Pearl River (see our argument below) but temperature and time of year may also play a role. One way color-matching is achieved in salamanders is by keeping the melanin in melanophores in punctate condition, which is quite obvious in the holotype and paratypes from Talisheek Bay, Louisiana (Fig. 6a,b and 7a). To color match a blacker substrate, the pigment granules need only to be dispersed in the melanophores, which is why populations east of the Pearl River are generally darker on blacker decomposing plant matter without substantial loess. The female in Fig. 7b with the darker belly is from the Strengthford, Wayne Co. site, which has moderately darker soils. Juveniles retain vestiges of the larval pattern of 6–8 pairs of faint, reddish-brown mid-dorsal blotches set off by fringing black edges that coalesce dorsolaterally and are more scalloped out onto the top of the tail (Fig. 10e, 11a). The juvenile middorsal color is often dark reddish brown but lightens to a bright red-orange color on the top of the tail (Fig. 11a). This brighter orange-red color on the top of the first half of the tail is a diagnostic feature of *D. valentinei* and can usually be seen in darker specimens (Fig. 11b), although it is not always so prominent in every specimen (Fig. 11c). All these more discrete aspects of the juvenile color pattern fade as individuals age. The color pattern and general morphology of adult females is similar to that of adult males (Figs. 6b, 7a). Old adults of both sexes tend to lose much of the juvenile pattern and become more-or-less uniformly patterned (Fig. 6a,b).

Sexual dimorphism. *D. valentinei* exhibits sexual dimorphism in the following characters: adult males are larger than adult females; males possess a mental gland at the tip of the lower jaw; the ventral opening in adult males is proportionately longer than in females, possesses papillose cloacal lips, and is usually outlined with melanin. Males also have a proportionately larger head, longer snout, and more massive quadrato-pectoralis muscles that give the adult males a "jowly" appearance (Schwenk and Wake 1993). The teeth of the premaxillary bone are monocuspidate, longer, and more pointed in adult males (Fig. 4b) for use in scratching the dorsum of the
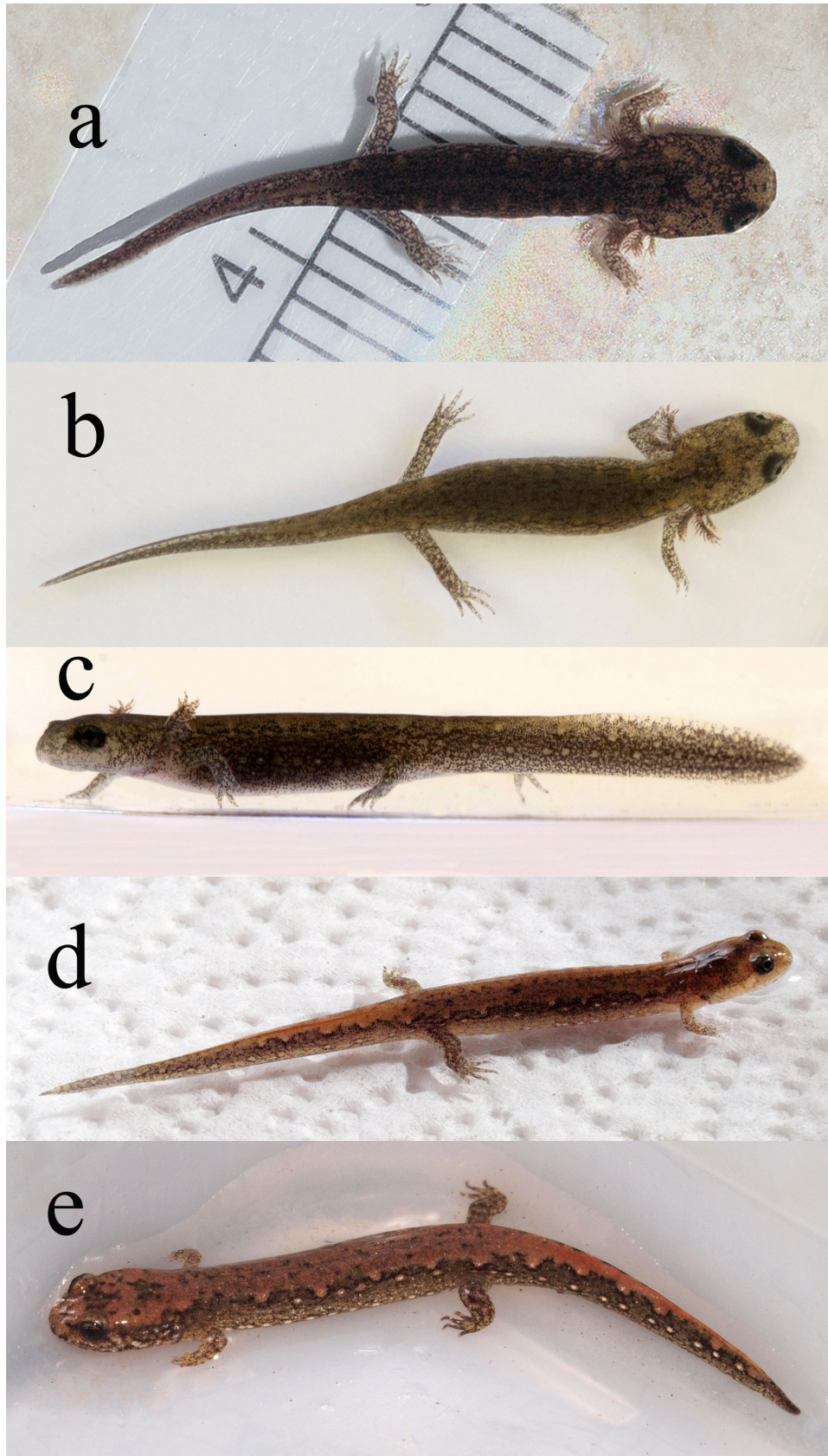
FIGURE 10. a = hatchling from the clutch of eggs in Fig. 9 (scale = 1.0 mm); **b, c** = dorsal and lateral views of larva of *D. valentinei* **sp. nov.** from Camp Shelby, Forrest Co., MS (photographs used with permission from Daniel McNair); **d** = metamorphosed *D. valentinei* **sp. nov.** captured as larvae from Camp Shelby, Forrest Co., MS and raised in captivity (27 mm SVL); **e** = juvenile *D. valentinei* **sp. nov.** from Strengthford, Wayne Co., MS (25 mm SVL).

FIGURE 11. Variation in color pattern of *D. valentinei* sp. nov.: **a** = juvenile from Talisheek Bay, St Tammany Parish, LA; **b** = female from Strengthford, Wayne Co., MS (MMNS 19447); **c** = male from near Grand Bay, Mobile Co., AL (photograph by David Welford and Karl Studenroth).

FIGURE 12. Olive-brown muddiness of wetland habitat at Talisheek Bay, St. Tammany Parish, Louisiana (type locality) due to the presence of glacial loess mixed with decomposing plant matter.

female during courtship (Organ 1961, Arnold 1977, Lamb in press). In adult females, enlarged ovarian ova can be seen as light yellow coloration through the skin of the lower belly and through the skin of the sides of the posteriormost five or six costal folds just in front of the insertion of the hind limbs (Fig. 7a).

Habitat and associates. Comparing what were at the time identified as *fuscus* (*conanti* [sensu lato]) and *auriculatus* (*D. valentinei*), Valentine (1963) concluded that the two species were sympatric throughout southern Mississippi with some evidence of ecological separation, *conanti* (sensu lato) preferring cooler habitats and running water such as might be found in shaded hillside streams while "…*auriculatus* [*valentinei*] is an inhabitant of muddy, bottomland swamps and sloughs." However, as evidence that the two were not environmentally selected phenotypes, he reported finding both species side by side at 12 collection sites. Recent surveys by J. Y. Lamb and J. R. Lee have confirmed that *Desmognathus valentinei* occurs syntopically with another species of *Desmognathus* at the Camp Shelby site in Forrest Co., MS, and J. Y. Lamb has found *D. valentinei* and *D. conanti* (sensu lato) within the Perkins Creek drainage, Lamar Co., MS. Our habitat data for *D. valentinei* are consistent with those of Valentine (1963) and indicate that "swampy, muddy, bottomland swamps and sloughs" are its principal habitat. Where we have found both species together, or at least near one another within the same drainage, is where hillside ravine and seepage-fed habitats flow into bottomland swamps of the larger streams and rivers.

Valentine (1963) mentioned that specimens he identified as *D. auriculatus* (*D. valentinei*) from eastern Louisiana were pale but gave way to darker populations moving east across the Pearl River into Mississippi. He also stated that the dark form that occupied southeastern Mississippi was usually paler than Gainesville, Florida specimens. *Desmognathus valentinei* is, indeed, paler overall and we propose an hypothesis to explain why. During more than half a century of fieldwork in wetland habitats of the southeastern U. S. Coastal Plain, we have been impressed by the light gray to tan muddiness of swampy bottomland sites and soils in southern Mississippi and the Florida parishes of Louisiana (Fig. 12) as opposed to the black muckiness of soils in similar wetland sites farther east. The lighter color of these muddy soils is the result of glacial loess that accumulated to the west and especially east along the Mississippi River during the late Pleistocene (Heinrich 2008). Heinrich's (2008) loess map of Louisiana and Mississippi shows that along the eastern valley sidewall of the Mississippi River nine meters of loess thins out to less than one meter at the Pearl River in Louisiana and Mississippi and then drops off eastward.

Heinrich's loess map was created from surface sampling, but silt particles are more likely to be deeper or more prevalent in bottomlands where they accumulate by sheet flow off the land. We propose that the light gray to tannish ground color of *D. valentinei* results from color-matching of loess in wetland substrates. Desmognathine salamanders have long been known for colors and color patterns that match the substrates they live on, and they are capable of metachrosis, the process of actively changing color over a few minutes to hours (Means 1974).

The vegetation of most of the swampy habitats of *D. valentinei* has changed dramatically during post-settlement times from extensive logging. In the pre-settlement vegetation, and in recovering sites today, the principal overstory trees include swamp black gum (*Nyssa biflora*), swamp tupelo (*N. aquatica*), sweetbay (*Magnolia virginiana*), bald cypress (*Taxodium distichum*), red maple (*Acer rubrum*), green ash (*Fraxinus pennsylvanica*), sweetgum (*Liquidambar styraciflua*), swamp laurel oak (*Quercus laurifolia*), and others.

Some herpetological ecological associates include the dwarf salamander (*Eurycea quadridigitata* complex), southern two-lined salamander (*Eurycea cirrigera*), three-lined salamander (*E. guttolineata*), mud salamander (*Pseudotriton montanus*) (Brown and Lamb 2016), lesser siren (*Siren intermedia*), one-toed amphiuma (Brown and Lamb 2016), two-toed amphiuma (*Amphiuma means*), and glossy crayfish snake (*Regina rigida*).

Reproduction. Lamb (in press) used time-lapse photography to describe some of the courtship behaviors exhibited by populations of *D. valentinei* and *D. conanti* (sensu lato) from the Pascagoula and Pearl River drainages in Mississippi. The most notable difference between the courtship behaviors exhibited by *D. valentinei* and those of many other species of *Desmognathus* (Verrell 1999) is that individuals of *D. valentinei* perform the "waltz" behavior frequently within courtship encounters and for extended periods of time ($\bar{x}$ = 2.5 min, range = 1–7 min) (Lamb in press). The waltz was first described by Verrell (1997) from populations of what he identified as *D. auriculatus* (*D.* cf. *auriculatus*) in South Carolina. Lamb's (in press) work, in conjunction with the molecular evidence presented here and in Beamer and Lamb (2008), indicate that the waltz behavior occurs across at least two, divergent lineages of *Desmognathus*. More detailed courtship ethograms, based on continuous footage and or observation, are needed for *D. valentinei*, as well as for other *Desmognathus* in the Coastal Plain.

James R. Lee found a female *D. valentinei* (62 mm SVL, 124 mm total length) with 11 recent hatchlings beneath a log that was lying flush against muddy substrate at the Camp Shelby site on 27 October 2014. Of the 6 hatchlings that were collected and raised in the laboratory, 3 successfully metamorphosed on 12 February 2015, 108 days post-collection (Fig. 10d). Two clutches of eggs were oviposited by *D. valentinei* in the laboratory (Lamb in press). One female *D. valentinei* (MMNS-19446) oviposited a single clutch on 7 October 2014 (N = 31 eggs) after storing sperm for at least 127 days, and the same female oviposited another clutch on 15 September 2015 (N ≥ 27 eggs) after storing sperm for 149 days (Lamb in press). It took this female >24 hours to complete oviposition for each clutch. The first clutch began hatching on 1 December 2014, and all 31 individuals hatched within 56–59 days post oviposition (Figs. 9, 10a). Only nine of the eggs in the second clutch appeared to have been fertilized, and all 9 individuals hatched 46 days post oviposition (Lamb in press). The 31 hatchlings from the first clutch (2014) were raised in captivity, and all successfully metamorphosed after 70–90 days. All larvae were fed a mixture of food items (e.g., frozen bloodworms, live zooplankton), maintained at a density of ca. 3–4 larvae per 1.5 L of water, and kept in an environmental chamber set to a 12:12 day: night cycle and constant temperature of 21˚C.

Larvae were found in the field on 11 and 12 January 2016 ($\bar{x}$ = 20.6 mm SVL; N = 4) and 16 March 2016 ($\bar{x}$ = 25.5 mm SVL; N = 2) from a site in Harrison Co., MS. Metamorphosing individuals with small dark gill stubs in Southeastern Louisiana University's museum were collected on 21 January 1950 from a population in St. Tammany Parish, Louisiana, (N = 10; SLU #06579), as well as on 25 February 1951 from a population in Jefferson Parish, Louisiana (N = 2; SLU #06605).

Geographic distribution. The geographic distribution of *D. valentinei* includes the localities for all the Mississippi specimens examined and mapped by Valentine (1963) in the inset of his Fig. 4 from Amite, Carroll, Covington, Forrest, George, Hancock, Harrison, Hinds, Jackson, Jasper, Jefferson Davis, Jones, Lamar, Lauderdale, Neshoba, Pearl River, Rankin, Scott, Smith, Stone, Wayne, and Wilkinson counties. This distribution includes the Lower Mississippi, Big Black, Amite, Bogue Chitto, Pearl, and Pascagoula drainages and at least the Bogue Chitto and Pearl drainages in the Florida parishes of Louisiana. In addition, the species may still range further east into Baldwin and Mobile counties, Alabama (Figs. 5, 11c). The current, westernmost extent of *D. valentinei* in Louisiana is uncertain. Museum specimens from three localities across Rapides (LSU 61053), Avoyelles (LSU 18387), and St. Landry (LSU 14416) Parishes, LA, were identified by D.B. Means as *D. valentinei* (Fig. 5), but the presence of other distantly related clades of *Desmognathus* in this general region necessitates further field and laboratory study (Lamb 2016).

Redescription of *Desmognathus auriculatus* (Holbrook, 1838)
Figs. 5, 13–19.

Suggested common name: Holbrook's Southern Dusky Salamander

Holotype. Undesignated, but a type illustration exists (Holbrook 1838; Fig. 13).

Syntypes. USNM 3901 and USNM 271136-45 (Dunn 1926).

Etymology. Named for the broad, reddish-brown stripe from the lower eyelid to the top of the posterior part of the upper lip which is a pleisiomorphic character for species of *Desmognathus*. Holbrook (1838) coined the specific epithet in reference to the "reddish-brown spot near the ear" and "an oblong reddish-brown spot behind the ear" (from the Latin words *auricula* = little ear and the ending *-atus* = provided with or having the nature of) in what is otherwise a generally black salamander. Of course, salamanders do not have ears, per se.

Diagnosis. In comparison with other *Desmognathus* in the Gulf Coastal Plain, adult *Desmognathus auriculatus* are black to very dark olive brown over their entire bodies (Fig. 14a,b,c) and sometimes overwashed with red pigment (Fig. 15a,b). The sides are uniformly dark and not lighter ventrolaterally (Fig. 14b) as in other Coastal Plain species such as *D. apalachicolae, D. conanti, D. monticola,* and *D. valentinei*. The belly is unmistakably black but may be densely peppered with small whitish or silvery speckling (Fig. 14c). *D. auriculatus* has a laterally compressed, bladelike tail with a distinct dorsal keel (Fig. 14a, b) as opposed to round and terete tails continuously narrowing in diameter to the tip such as in *D. apalachicolae* or trigonal in other *Desmognathus* within the Coastal Plain. Individuals of *D. monticola* (Dunn) are larger, but *D. auriculatus* is larger than *D. apalachicolae*. Juveniles of *D. auriculatus* are coal black dorsally and do not display a series of brightly colored alternating or paired dorsal round blotches that are always present on juveniles and usually females of *D. monticola, D. apalachicolae*, and *D. conanti* (sensu lato).

Description. Live juveniles and adults of Holbrook's Southern Dusky Salamander, *D. auriculatus*, in Florida and Georgia are coal black dorsally, ventrally, and laterally (Fig. 14) but some degree of reddish pigment is often present (Fig. 15). The reddish pigment is most common on the back, on top of the basal part of the tail, in the classic desmognathine patch from the posterior angle of the eye to the corner of the mouth, and overlying the lines of round portholes or light spots of the neuromast vestiges, but never on the belly. Some populations living on white sandy substrates of spring boils such as Silver Glen Springs in central Florida appear quite reddish because of an abundance of this reddish pigment overlying the basic black ground color (Fig. 15a,b), but populations living on black, decomposing organic matter in blackwater swamps may have very little reddish pigment (Fig. 14c).

The basic ground color of the belly of *D. auriculatus* is black, but peppered with numerous white or silvery specks (Fig. 14c). The sides of *D. auriculatus* are black and may have some of the same white specks as the belly, but almost always have a pronounced row of lighter colored, often reddish, portholes between the armpit and groin (Figs. 14b, 15a,b). These ventrolateral large round spots are more pronounced in *D. auriculatus* than in other sympatric *Desmognathus*, and when they are bright red, they are diagnostic. The portholes are also prominent in allopatric *D. valentinei*, but not overwashed with red color. The two dorsolateral lines of light spots may or may not be present, but the middle one of the three lateral lines of light spots usually runs out along the sides of the tail and is obvious. Ventrolaterally, where the sides of the body turn under and become the belly, there is no strong contrast of dark lateral pigment versus a lighter colored belly. In most other Coastal Plain *Desmognathus*, however, the pigment of the sides of the body is two-toned, being darker dorsolaterally and lighter ventrolaterally.

The tail is decidedly bladelike all the way to its tip. It is 2 to 3 times deeper (dorsoventrally) than wide at a point two-thirds of the way distally from the vent. The depth is due to a fleshy dorsal ridge. In other species the tail may taper continuously from the base to the tip and be round throughout (e.g. *D. apalachicolae*) or trigonal, meaning laterally compressed with the dorsal half narrower than the ventral half. However, the tail is nowhere deeper than wide in other species (e.g. presumptive *D. conanti* and *D. monticola*).

Interspecific differences exist in average body size of mature males and females of all four described Coastal Plain species of *Desmognathus*. Mature males of Coastal Plain *D. monticola* from Florida averaged 58.2 mm SVL (range 43.9–60.7, N = 13) and mature females averaged 56.8 mm SVL (range 48.8–60.7, N = 6) (Means and Longden 1970). *D. monticola* is the largest of all the Coastal Plain *Desmognathus*. Mature male *D. apalachicolae* averaged 46.3 ± 3.49 mm SVL (range 40.0–51.8, N = 30) and mature females averaged 38.5 ± 3.98 mm SVL (range 33.0–46.9, N =27) (Means and Karlin 1989). A Florida population of *D. conanti* (sensu lato) had males that averaged 41.0 ± 2.23 mm SVL (range 37 .7–44.7, N = 10) and females that averaged 38.5 ± 1.27 mm SVL (range

33.0–38.1, N = 13) (see Means and Karlin 1989 under *D. f. conanti*). The ranking of Gulf Coastal Plain *Desmognathus*, from largest species to smallest, is *D. monticola* > *D. valentinei* > *D. auriculatus* > *D. apalachicolae* > *D. conanti* (sensu lato).

A series of 8 hatchlings (collected as eggs on 3 October 1955 and hatched on 7 October) from Alachua Co., FL (UF 34689 to 34695), measured 10.0 ± 1.31 (range 9.1–13.1) mm SVL and 15.4 ± 1.78 (range 14.4–19.8) mm total length. A series of 14 larvae collected at different times from the same locality averaged 17.3 ± 3.81 (range 11.5–23.6) mm SVL and 27.6 ± 5.92 (range 19.0–36.8) mm in total length. Likewise, 6 metamorphs from Devil's Millhopper collected over different dates averaged 24.3 ± 3.05 (range 21.2–29.2) mm SVL and 43.2 ± 3.07 (range 39.4–47.2) mm in total length. Nine metamorphs collected 12 May 1998 and about one month old from Bradwell Bay, Wakulla Co., Florida measured 24.4 ± 2.19 mm SVL and 42.0 ± 4.2 mm in total length.

FIGURE 13. Scan of type description of *Desmognathus auriculatus* from Holbrook (1838).

FIGURE 14. a = adult male; **b** = adult female *Desmognathus auriculatus* from Ft. Stewart, Bryan Co., Georgia, near the type locality. Note longer snout in male; both may have regenerating tail tips; **c** = belly of male in "a" above. Note the small mental gland.
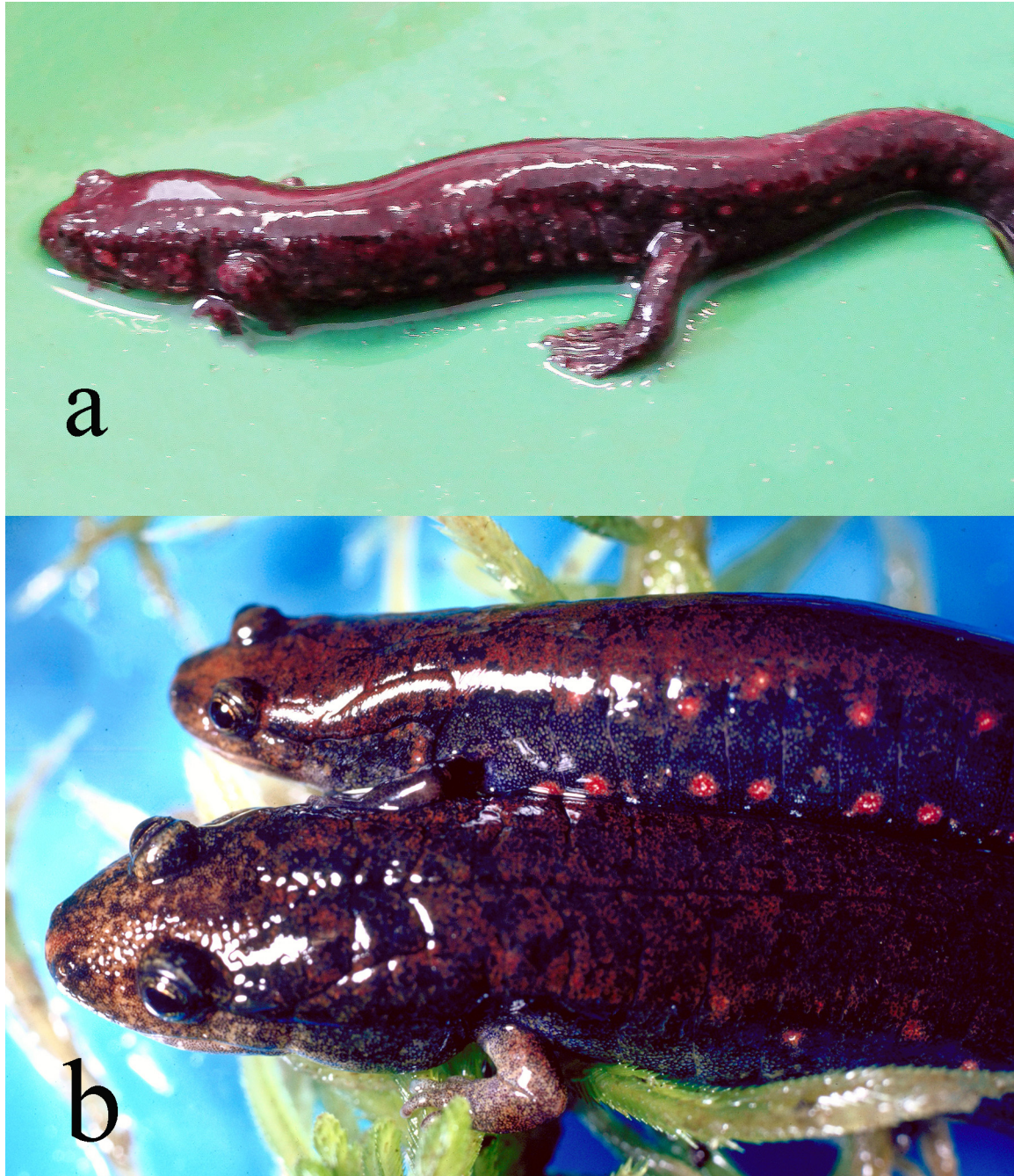
FIGURE 15. Dorsal coloration of *D. auriculatus* overwashed with reddish pigment from **a** = Tates Hell Swamp, Franklin Co., FL (photograph courtesy of Diane Alix) and **b** = gravid female above and adult male below from Deep Springs Canyon (steephead), Bay Co., FL.

Season of Courtship. Courtship in *Desmognathus auriculatus* has not been reported. Verrell (1997) described courtship behaviors from laboratory observations with populations from Aiken and Barnwell counties in South Carolina, but these are outside of the range of *D. auriculatus* according to the DNA study of Beamer and Lamb (2008) and the data presented herein. Courtship in *D. auriculatus* probably takes place in the spring and summer months, most likely during the season when gravid females with enlarging ovarian ova > 2.0 mm in diameter are present in the field.

Seasonal Occurrence of Gravid females. The sample of 195 females plus Richard Highton's sample from Devil's Millhopper in Alachua Co., Florida was dissected and scored for the diameter of ovarian ova (Fig. 16). Although females with enlarging ovarian ova up to 2.0 mm in diameter were found in nearly all months of the year, females with yolking ova > 2.0 mm occurred over a 28-week period from 26 January to 26 August (Fig. 16).
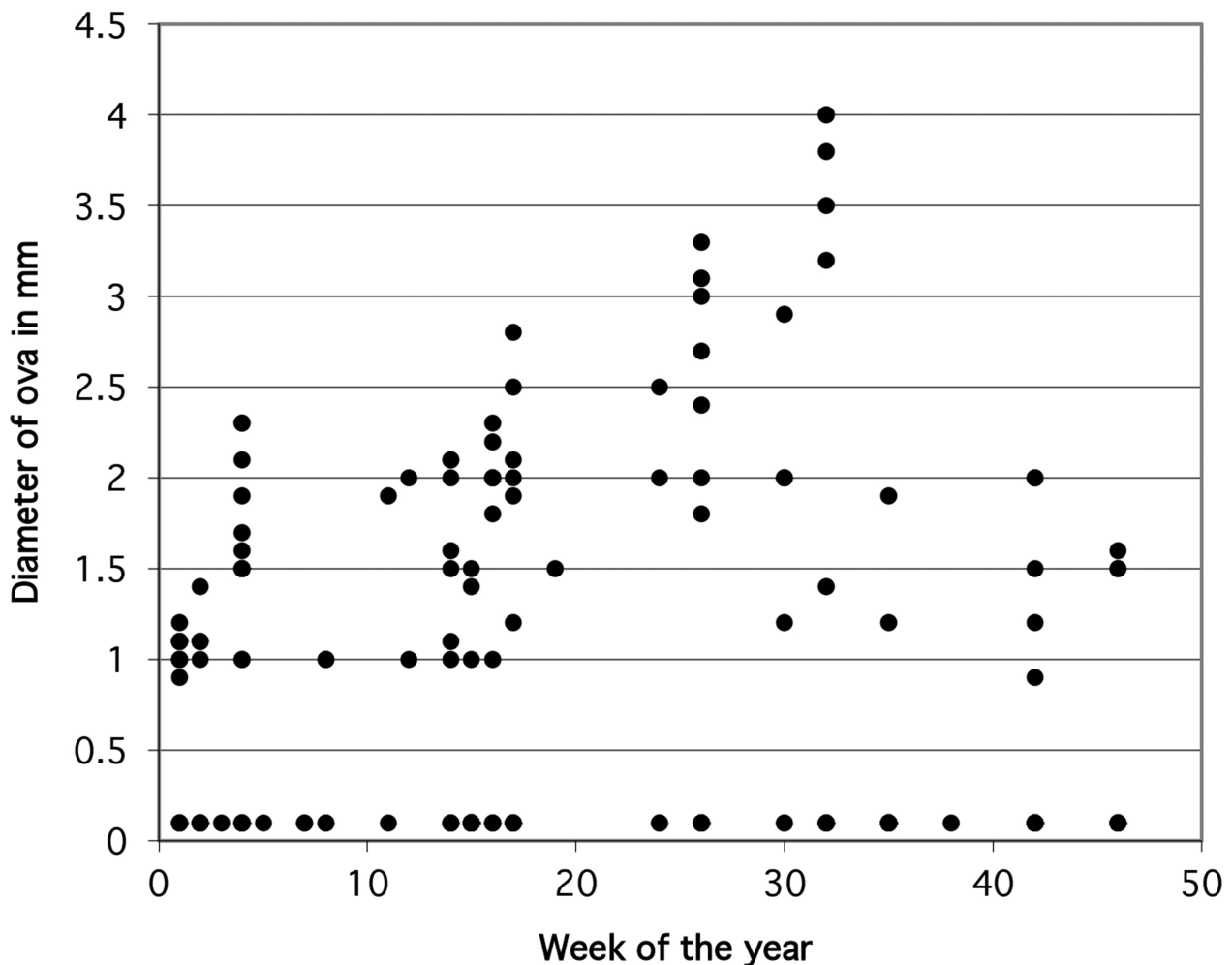
FIGURE 16. Ova diameters by week of the year. Note that ova > 2.0 mm diameter first appear in the last week of January, increase through the spring and summer, then disappear after the last week in August, when eggs are laid in nature. Females yolking ovarian ova (1.0–2.0 mm diameter) occur throughout most of the year. The gap in data points between 0.1 and 1.0 mm diameter is an artifact of measurement; ova smaller than 1.0 mm were too small to measure so were assigned the value 0.1 mm (as most of the undeveloping ova truly were).

Season of Oviposition. Oviposition is not a behavior easily observed in nature, but the timing of oviposition can be bracketed by when gravid females are spent and when females guarding eggs become present in field observations and in preserved collections. The end of the period when gravid females have been observed in the field is the second week in September. The first eggs of *D. auriculatus* that have been found in the field were on 12 September. Oviposition, therefore, must begin at least by 12 September and probably continues into early October.

Eggs. Eggs have been observed in nests with their brooding mothers in Florida between 12 September and 14 November (Fig. 17). Dates on which female *D. auriculatus* were found brooding eggs in the field in Florida were 26 September 1968 (Wakulla Co., DBM-1140), 3 October 1955 (Alachua Co., UF-34689), 3 October 1969 (Okaloosa Co., DBM-1223), 22 October 1969 (Leon Co., DBM-1229), 6 November 1974 (Bay Co., DBM-1957), and 14 November 1971 (Bay Co., DBM-1620).

Hatchlings and larvae. Hatchling *D. auriculatus* emerged on 7 October from eggs that were collected on 3 October at Devil's Millhopper, Alachua Co., Florida. Other hatchlings from the Alachua Co. site were found through 10 December. Apparently hatchlings can be found over at least a two-month period from early October to early December. In the 32 times that older larvae were found in the field, the earliest date was 17 December and the latest date was 8 May, a period of 142 days. However, hatchlings and older larvae from Devil's Millhopper, Alachua Co., Florida, were present from 3 October through 19 August, a period of 322 days. Assuming that the latest metamorphosing larvae are from the cohort of latest hatchlings, the maximum larval life of *D. auriculatus*

would be 322–62 = 260 days or about 8.5 months. However, observations made over the past decade on the population in Bradwell Bay, Wakulla Co., Florida, indicated that *D. auriculatus* larvae have the capability of metamorphosing earlier if faced with the threat of desiccation as larval habitat dries up. Nine larvae of *D. auriculatus* averaged 19.1 mm SVL (31.1 mm total length) and were coal black to the naked eye (Fig. 18). The hind legs are twice as robust as the front legs (a characteristic of the genus at all life stages) and three bushy external gills are prominent at the sides of the neck. The gill rami have black melanophores on them, but appear reddish from their blood supply. The bladelike larval tail is about as long as the rest of the body and has a pronounced dorsal fin to the tip (Fig. 18). Neuromast organs of the three lateral lines are surrounded by circles of white iridophores. These are most prominent on the ventrolateral line between the armpit and groin and the two dorsolateral lines that extend out onto the sides of the tail (Fig. 18).

FIGURE 17. Female *Desmognathus auriculatus* guarding her eggs, 26 September 1968, FL, Wakulla Co., Wakulla River at Upper Bridge. Under log in soft, peaty/sandy sediment on stream shore.

FIGURE 18. Larva of *Desmognathus auriculatus* from Bradwell Bay Wilderness Area, Wakulla Co., Florida.

In three different years at the Bradwell Bay locality, we found metamorphs in April (17 April 1999; 15 April 2000) and May (12 May 1998), but no larvae were collected on 8 March 2000 although there was plenty of water in the habitat. In this case the absence of larvae may be because the swamp had been completely dry until a few days before our visit, as were most of the isolated wetlands in the region at that time due to a prolonged drought. The water we encountered was the result of a recent heavy rain. Either embryos in eggs had died from the drought before the rain, or water levels had not risen into the peat islands where the eggs could hatch and larvae find the water.

Metamorphs. Judging from gill nubs found at the sides of the neck, we collected recently metamorphosed *D. auriculatus* on 7 dates between 9 April and 7 July. Metamorphs were collected from Devil's Millhopper in Alachua Co., Florida, on 4 dates between 10 April and 6 June. Metamorphs look like larger juveniles and adults.

Geographic distribution. Because genetically validated samples have been collected from the Ogeechee Basin in southeastern Georgia (EU311680), to the Suwannee drainages of south-central Georgia and Florida (EU311650, EU311681), and the eastern Ochlockonee Basin in the eastern Florida Panhandle (JYL268–270; this study), we assign populations that inhabit the Altahama and St. Mary's drainages of south-central Georgia and thence south through peninsular Florida to the Alafia River (Krysko *et al.* 2011) to *D. auriculatus* (Fig. 5). This accords with prior hypotheses (Means 2008, Graham *et al.* 2010, Beamer and Lamb 2008, Maerz *et al.* 2015) with the exception that genetic data from a separate study indicate that presumptive *D. auriculatus* populations in the western Savannah River Drainage are likely an undescribed, phylogenetically distant and convergent taxon (Bernardo *et al.* unpublished data).

Previous studies based on morphological comparisons, and the osteological comparisons included here, suggest that *D. auriculatus* once ranged farther west to the Yellow River and steepheads in the southwestern part of Eglin Air Force Base (Means 1974, 1975, 1999, Means and Travis 2007) and in Alabama from the southeastern tier of counties bordering the Florida panhandle (Means 2004, 2005; Graham *et al.* 2010). Unfortunately, many populations in Florida (Dodd 1998), Georgia, and Alabama (Graham *et al.* 2010, Maerz *et al.* 2015) have declined or have been extirpated, precluding molecular analyses and resolution of their evolutionary affinities. This is especially true for the Florida panhandle where more than 65 robust populations prior to the mid-1970s had completely disappeared by the late 1990s (Means and Travis 2007).

Habitat. When *D. auriculatus* was easily found in the Coastal Plain in the 1960s and early 1970s, its prime habitat was the swampy backwaters of river floodplains such as the Ochlockonee, Wakulla, and Satilla rivers in north Florida and south Georgia. Swampy tributary streams of the Ochlockonee, Apalachicola, and Suwannee rivers were equally if not more productive. The peaty margins of swampy lakes such as Lake Iamonia and cypress-dominated lakes in southern Leon Co., Florida, were also good habitats. Bay-gall communities associated with sluggish flatwoods streams within 50 miles of the Gulf Coast were good *D. auriculatus* habitat as well as the wet, swampy portions of Florida's spring-fed rivers such as those issuing from Silver Glen Springs and Juniper Springs in Marion Co. and Wakulla Springs in Wakulla Co.

Other prime habitats for *D. auriculatus* were large, depressional basins in the coastal lowlands that, during rainy periods, are drained by sluggish streams that spread out over the basins covering hundreds and even thousands of acres with swamp waters containing dissolved organic acids. There are numerous such basins on the Osceola and Apalachicola national forests, for example, and such swampy basins occur all around the margins of the Atlantic and Gulf Coastal Plain. The Okefenokee Swamp on the Florida/Georgia border and Bradwell Bay in Wakulla Co., Florida, are good examples (Fig. 19). In fact, because *D. auriculatus* inhabits these swampy

lowlands, Means (1974, 1975) hypothesized that gene flow in this species occurred relatively unimpeded among these swampy coastal lowlands. That is why Means (1975) argued that *D. auriculatus* was able to colonize ravine habitats and called steepheads in certain Florida western panhandle drainages because these drainages did not have connections to major river systems that might have enabled desmognathans with northern affinities (D. *apalachicolae, D. monticola, D. conanti* [sensu lato]) to disperse into them.

FIGURE 19. Dark reddish-brown to black muck and peat of wetland habitats without glacial loess. Bradwell Bay Wilderness Area, Wakulla Co., Florida, where 15 individuals of *D. auriculatus* were scraped upslope from the water's edge and caught as they wriggled back to the water.

Devil's Millhopper in Alachua Co., Florida was a ravine-like habitat in which *D. auriculatus* occurred in the absence of a congener (Dodd 1998). The species may also have been common in steepheads along the Atlantic side of the Florida peninsula such as those in Gold Head Branch and Palatka Ravines state parks, but when this possibility was investigated in the late 1970s, the decline of *D. auriculatus* was already well underway or had taken place (Means and Travis 2007) and no individuals were found.

Desmognathus auriculatus was never found in any Coastal Plain ravine of classic gully-erosion origin. Most of the ravines in the Coastal Plain are of this origin, including all the ravines along the major rivers transecting the Coastal Plain (Lower Mississippi, Pearl, Leaf-Pascagoula, Alabama-Tombigbee, Escambia-Conecuh, Choctawhatchee, Apalachicola-Chattahoochee, Savannah). We were unable to obtain DNA from any population between the Apalachicola and Alabama-Tombigbee rivers along the eastern Gulf Coast, but in the 1970s, Means (1974, 1975) collected many samples of what he thought were *D. auriculatus* from special ravine habitats called steepheads in the Florida panhandle. These populations were identical to swamp-inhabiting and topotypical populations in color pattern, body size, bladelike tails, and severely reduced premaxillary fontanelle of 8 individuals from 3 steepheads west of the Choctawhatchee River. Steepheads appear to be confined to the Florida panhandle and northeast Florida. For a more detailed description of the steephead habitat see Means (1975, 2000) and Means and Travis (2007).

Microhabitats. Microhabitats occupied by metamorphosed juveniles and adults of *D. auriculatus* for localities in Florida and Georgia were from under logs in very soupy-mucky parts of floodplains; buried in very wet muck

while raking for *A. pholeter*; from under leaf packs and debris at mucky depressions; edge of muck in depressions fed by spring-seepage; at the air/water/soil interface under logs at the edge of water in peat-bottomed gum swamps; in mucky potholes in the bed of a drying creek; under logs in low, wet mucky sites and under an inch of rotting leaves lying in water. Fig. 19 shows prime, swampy flatwoods habitat of a gum swamp (all three *Nyssa* spp. were present) with the water's edge microhabitat of *D. auriculatus* exposed after leaf litter was scraped upslope in Bradwell Bay Wilderness Area, Wakulla Co., Florida.

All of the above habitats and microhabitats were associated with lotic systems, rivers and streams. Lentic habitats of lakes and ponds are hydrologically different from lotic habitats, but *D. auriculatus* never-the-less inhabited them as well when lake margins were dominated by decomposing organic matter. The microhabitat along lake margins was found to be as follows: 1) very edge of Lake Iamonia several inches under the peaty-muck of decomposing vegetation; 2) underneath decaying leaf litter of bottom sediments of a small cypress pond in Leon Co., Florida, which had dried up; the salamanders were about an inch under the surface but retreated downward into wetter muck below the water table when disturbed. Larvae were collected in the same microhabitats as when adults were found lying in shallow water.

Nest sites. Egg clutches and brooding females were recorded in the following microhabitats: 1) brooding female and one clutch found under sphagnum moss in a seepage area; 2) brooding female found coiled around a clutch of eggs under a board lying on a site with saturated black peat (Fig. 17); 3) under sphagnum moss in a seepage area with attendant female.

Behavior. *Desmognathus auriculatus* is well equipped to actively burrow into the peaty sediments of its microhabitats. Of all *Desmognathus, D. auriculatus* has the skull morphology most adapted for burrowing. Burrowing behavior in obvious attempts to escape was observed almost every time a specimen was collected. Horizontal movements correlated with shifting wetland habitat were deduced from the following observations. In Bradwell Bay Wilderness Area water stands or very slowly runs off the shallow basin into Monkey Creek from large (up to 15 m in diameter), interconnected pools. During periods of normal rainfall, water stands no more than about 50 cm deep in the pools, but then gradually drains away or evaporates exposing the peaty bottom sediments in seasonal droughts. On at least seven occasions (12 August 1983, 5 November 1989, 7 June 1997, 8 March 2000, 12 November 2001, 20 January 2002, 20 March 2002) when water levels were high (60–120 cm deep), specimens of all age classes were found at the water's edge but could not be dipnetted from water deeper than about 10 cm. On other occasions when water levels were down and bottom sediment peat was exposed, *D. auriculatus* was again very abundant right at the water's edge (Fig. 19), but not present under peat or logs upslope even 30 cm away from the water's edge. During droughts when only a few shallow puddles were found throughout the whole swamp (26/10/85, 26/04/92, 12/05/98, 17/04/99), *D. auriculatus* was abundant at the receded water's edge but not at all upslope where it had been abundant when water levels were up. On 06/06/01 the swamp was completely dry and no specimens of *D. auriculatus* were found in four person-hours of effort, and yet five months later on 12/11/01, about 50 juveniles and a few adults were found in about three person-hours of effort in the identical sites (Fig. 19). When individuals were exposed under logs, they usually attempted to escape down crayfish burrows in the peat.

To test whether *D. auriculatus* makes vertical movements in the peat during severe drought, on 20/04/85, a 45 cm wide X 90 cm long trench was dug down into the peaty bottom of a dried pool in which salamanders had been previously abundant but on that day were not present near the surface. At the bottom of the peat, about 50 cm deep, an adult, two juveniles, and two larvae with nubs for gills were excavated from little tunnels at the bottom of the peat just above wet, black sand.

We estimated the density of *D. auriculatus* in prime swampy habitat in the Bradwell Bay habitat, Wakulla Co., Florida. From the edge of one drying pool whose circumference was ~30 m (~10 m in diameter), 23 juveniles to small adults were collected, or 23/30 = 0.77 individuals/linear meter. This approximate number was found throughout the swamp at the edge of other drying pools. Most of the salamanders collected around the margins of drying pools were metamorphs or juveniles of the past year or two, however. Large, sexually mature adults were rarely found in this microhabitat. They were most abundantly found under the heaviest logs and under the most substantial woody cover at water's edge, but sometimes upslope a short distance. The microhabitats safest from predators appear to be controlled by the adults who may keep juveniles away by aggressive behavior. Probably the microhabitats most occupied by adults is one that has not been well sampled. It is the moist, peaty soil of the brushy islands in the swamp that are densely packed with live roots of shrubs and trees, making digging into them nearly impossible. Burrows in these peaty/rooty sites, however, are probably where the bulk of the adults live and where

females brood their eggs. This may be why egg clutches of *D. auriculatus* in the Bradwell Bay locality have not been found in spite of the large numbers of juveniles that normally occur in abundance around the water's edge of receding pools.

The diet of *D. auriculatus* is poorly known and needs study. It probably consists of terrestrial invertebrates, considering that other *Desmognathus*, except *D. marmoratus*, primarily feed upon terrestrial invertebrates (Petranka 1998). After keeping a larva and medium-sized adult in the same plastic bag in a refrigerator for 48 hours, the adult regurgitated the larva, indicating that cannibalism of the young by adults may take place in nature (DBM-3043).

Amphibian and reptile ecological associates. We tabulated the number of amphibian and reptile species that were collected with *Desmognathus auriculatus* from localities in Georgia and peninsular Florida from 1968–2002. The amphibian and reptile associates of *D. auriculatus* in swampy habitats were species that are endemic, or nearly so, in Coastal Plain swampy habitats. The most abundant of these were salamanders, *Pseudotriton montanus*, *Eurycea quadridigitata*, *E. guttolineata*, and *Amphiuma pholeter*, but the list includes 13 other amphibians and 5 reptiles. One salamander, *Stereochilus marginatus*, would have been scored more often, but the sample of collections only included one locality in the range of the species.

Acknowledgments

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