Morphological Variation among Populations of the Western Slimy Salamander on the Edwards Plateau of Central Texas

Drew R. Davis[1,2] and Gregory B. Pauly[1,3]

We conducted a morphometric analysis on Western Slimy Salamanders, *Plethodon albagula,* from each of the five mitochondrially defined groups that occur on the Edwards Plateau of central Texas. Although several groups are similar in body size and/or shape, multivariate analyses do find significant differences among groups, and several groups have clear differences for one or several characters. Thus, for several between-group comparisons, the morphological data are consistent with the mitochondrial data in arguing for cryptic lineages of slimy salamanders on the Edwards Plateau. These results demonstrate that despite the common interpretation of morphological conservatism in plethodontids, detailed morphometric studies can be used in taxonomic and evolutionary investigations of these salamanders. Lastly, male central Texas *P. albagula* are found to have a larger mean body size than females; this pattern of sexual size dimorphism (SSD) is reverse from that observed in nearly all other plethodontids with SSD.

THE Western Slimy Salamander, *Plethodon albagula,* is a polymorphic species of lungless salamander with a disjunct range; it occurs on the Edwards Plateau of central Texas and over 380 km to the northeast in the Interior Highlands (i.e., the Ozark Plateau and Ouachita Mountains) of Arkansas, Missouri, and Oklahoma. The Western Slimy Salamander is the westernmost representative of the *Plethodon glutinosus* species complex, which is broadly distributed throughout much of the eastern United States (Highton et al., 1989; Conant and Collins, 1998). *Plethodon glutinosus* was originally recognized as a single variable species, but work by Grobman (1944) placed central Texas *P. glutinosus* into a separate subspecies, *Plethodon glutinosus albagula*. Later, species boundaries in the *P. glutinosus* complex were examined using allozymes. Defining species as sets of allopatric populations with a Nei's genetic distance (Nei's *D*) greater than 0.15, Highton et al. (1989) broke the *P. glutinosus* complex into 16 distinct species, including elevating *P. albagula* to full species status. This species criterion was contested (Frost and Hillis, 1990), but it was largely agreed that this complex consists of many historical lineages. Highton et al. (1989) also found a greater Nei's *D* among *Plethodon* populations on the Edwards Plateau (average $D = 0.11$) than among some Edwards Plateau and Interior Highlands populations (average $D = 0.05$); as a result, slimy salamanders from both regions were assigned to *P. albagula*.

On the Edwards Plateau, *P. albagula* occurs patchily in a relatively narrow corridor approximately 425 km long (Fig. 1). Despite this restricted distribution, mitochondrial DNA (mtDNA) sequence analyses uncovered multiple lineages within this region. Baird et al. (2006) identified five parapatric groups within central Texas that together form a clade to the exclusion of Interior Highlands *P. albagula*. The monophyly of three of these five groups was strongly supported (groups A, B, and D; Bayesian posterior probabilities [BPP] = 100%), and a fourth clade was also recovered with weaker support (group E; BPP = 76%). The final group (group C) identified by Baird et al. (2006) included several genetically similar haplotypes from geographically proximate populations in the southeastern corner of the Edwards Plateau; however, these haplotypes did not form a clade. Relationships among these five groups were not well resolved.

These DNA analyses suggest that there may be cryptic lineages of *P. albagula* on the Edwards Plateau. However, it is also possible for mtDNA phylogeographic breaks to exist without any underlying barriers to gene flow (Irwin, 2002). Data from additional markers (e.g., nuclear sequence data or morphological data) and/or comparative phylogeographic patterns can be used to infer whether the perceived mitochondrial break likely results from disjunctions in gene flow. In central Texas *P. albagula,* only the separation between groups A and B is coincident with an obvious long-term barrier to gene flow. These two lineages were separated by the Colorado River until a relatively recent (Pleistocene) southward shift in the river's location by several kilometers that isolated some lineage B populations north of the new river channel (Baird et al., 2006). However, a band of gravel and clay deposits, termed the Asylum Terrace, remains in the pre-Pleistocene location of the river; these deposits are unsuitable habitat for *P. albagula,* resulting in a continuation of the barrier to gene flow despite the river's shift (Baird et al., 2006). Given that there are no apparent barriers to gene flow among the remaining mtDNA lineages (e.g., between groups D and E or groups B and E; Fig. 1), it is possible that these mtDNA phylogeographic breaks are not associated with disjunctions in gene flow (*sensu* Irwin, 2002). Here, we conduct morphometric analyses on central Texas *P. albagula* to test whether there are morphological differences among these five groups. Such differences would support the hypothesis that there are true barriers to gene flow among these populations and multiple cryptic lineages on the Edwards Plateau.

Two additional goals of this study are to assess the utility of morphological characters for taxonomic studies of plethodontids and to examine patterns of sexual size dimorphism (SSD). Plethodontid salamanders, especially members of the *Plethodon glutinosus* group, have long been considered to be extremely morphologically conservative

[1] Section of Integrative Biology and Texas Natural Science Center, One University Station C0930, University of Texas, Austin, Texas 78712.
[2] Present address: Population and Conservation Biology Program, Department of Biology, Texas State University, San Marcos, Texas 78666; E-mail: drewdavis@txstate.edu.
[3] Present address: Department of Evolution and Ecology and Center for Population Biology, One Shields Avenue, University of California, Davis, California 95616; E-mail: gbpauly@ucdavis.edu. Send reprint requests to this address.
Submitted: 25 November 2009. Accepted: 20 October 2010. Associate Editor: S. A. Schaefer.
 DOI: 10.1643/CH-09-217

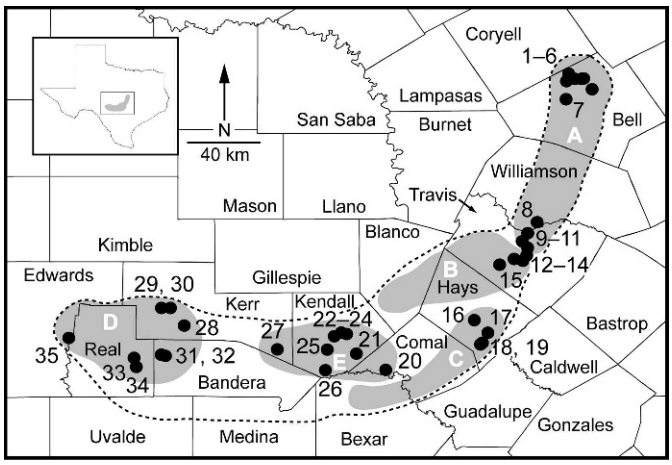

Fig. 1. Range map of *Plethodon albagula* in central Texas. The dashed line encircles the known range of *P. albagula* in central Texas. Gray shading depicts the range of each mtDNA group as identified by Baird et al. (2006). Collection localities for specimens examined in the morphological analysis are numbered and also listed in Table 1.

(Wake et al., 1983; Larson, 1984, 1989; Carr, 1996). Although casual comparisons among multiple species do suggest extreme morphological similarity, few studies have involved detailed morphometric analyses. Here, we will examine the occurrence of morphological variation at a relatively narrow scale—among geographically proximate populations of a single species. If variation exists at this narrow scale, then similar morphometric studies are likely to be informative for plethodontids at broader phylogenetic and taxonomic scales. Patterns of sexual size dimorphism have been previously characterized in many species of plethodontid salamanders, often to assess the impact of body size on male–male competition and/or reproductive success (Houck, 1988; Mathis, 1991; Bruce, 1993, 2000; Ryan and Bruce, 2000). In nearly all desmognathines, hemidactyliines, plethodontinines, and bolitoglossines in which SSD is observed, females are, on average, the larger sex (Bruce, 2000; Ryan and Bruce, 2000). However, patterns of SSD have not been examined in *P. albagula*.

MATERIALS AND METHODS

Sampling.—We examined morphometric variation in 106 adult specimens including 47 males and 59 females. This sample includes 18–23 individuals from each of the five groups identified by Baird et al. (2006). Within each group, individuals are from 4–11 collecting localities, with 19 localities and 20 individuals sampled in both Baird et al. (2006) and here (Fig. 1, Table 1). The presence of mental glands and cloacal papillae, which both only occur in males, was used to differentiate between males and females. Specimens were considered to be adults and therefore included in the analysis if they were larger than the smallest male in which a mental gland was visible. If females attain sexual maturity at larger sizes than males, it is possible that non-sexually mature females were included. However, such a scenario should only reduce our ability to identify sexually dimorphic characters.

Characters examined.—We measured the following 12 morphological characters: snout–vent length (SVL), distance from the tip of the snout to the anterior edge of the vent; head length, distance from the gular fold to the tip of the snout along the midline; head width, measured immediately posterior to the eyes; head depth, measured immediately posterior to the eyes; snout length, measured from the anterior angle of the orbit to the nostril; interorbital distance, measured from the inner edges of each orbit; orbit width, measured from edge to edge of the orbit; forelimb length, distance from the tip of the third finger to the insertion point of the forelimb; pectoral width, measured immediately posterior to the insertion point of the forelimbs; hind limb length, distance from the tip of the third toe to the insertion point of the hind limb; tail width, measured immediately posterior to the vent; and tail height, measured immediately posterior to the vent. Tail length is a commonly used character in morphometric studies, but we did not use it because many individuals had missing or regrown tail tips. The forelimb and hind limb characters were measured on the left lateral side. All characters were measured to the nearest 0.01 mm with a digital caliper. These are standard characters for assessing morphological variation in salamanders (Irschick and Shaffer, 1997; Pauly et al., 2007; Adams et al., 2009), and characters i, ii, iv, and v were also examined in Carr (1996).

Data analysis.—All analyses were conducted on log-transformed measurements. Some specimens had damage to portions of their body that prevented measuring certain characters. As a result, we lacked measurements for snout length for one individual, hind limb length for one individual, head length for six individuals, and tail width and tail height for three individuals. Missing values for these characters were estimated using linear regressions (Model I) against logSVL so that these individuals could be included in the multivariate analyses. Prior to conducting the regressions, we examined whether any of these five traits are sexually dimorphic across the entire sample using ANCOVAs with logSVL as the covariate and sex as a factor. Head length ($P < 0.001$), tail width ($P = 0.047$), and tail height ($P = 0.032$) were found to be sexually dimorphic so regressions to estimate missing values were performed separately for each gender. Snout length and hind limb length were not sexually dimorphic across the entire sample so all individuals were included in these regressions. This is a conservative approach as all individuals were included in the regression which could reduce among-group differences. All five characters were highly correlated with SVL in the regression analyses ($P < 0.001$).

To explore patterns of trait variation between males and females (i.e., sexual dimorphism) and among the five groups, we conducted regression analyses on the log-transformed measurements. Each individual's residual value was obtained from a pooled analysis that included all individuals for each trait. This approach was only used as an exploratory tool and not to factor out body size in the multivariate analyses.

We then explicitly tested for differences between males and females and among the five groups for each morphometric trait. Individuals with missing trait values were excluded from these analyses. Differences in SVL (i.e., sexual size dimorphism) were assessed using an ANOVA with sex and mtDNA-defined group (following Baird et al., 2006) as factors; differences in all other traits were assessed using an ANCOVA, again with sex and group as factors and logSVL as the covariate. To further examine sexual shape dimorphism, we used MANCOVAs of the cranial (head length, head

Table 1. Collection Localities, mtDNA Group Assignment Based on Geography, and Voucher Numbers of All Examined Specimens. Numbers in parentheses following localities are the collection locality numbers from Baird et al. (2006). Voucher numbers with asterisks were sequenced in Baird et al. (2006).

	County	Locality	mtDNA group	Voucher number
1	Bell	Lunch Counter Cave (22)	A	TNHC 62449*, 62452
2	Bell	Treasure Cave (20)	A	TNHC 61364*, 61365, 62450*
3	Bell	Buchanan Cave (24)	A	TNHC 62451*
4	Bell	Bear Spring (25)	A	TNHC 64164
5	Bell	Tweedledum Cave (21)	A	TNHC 63848*
6	Bell	Seven Cave	A	TNHC 62448
7	Bell	14.5 km NW of Belton, Mrs. Paulk's Ranch	A	TNHC 13845
8	Williamson	Chaos Cave (19)	A	TNHC 63855*
9	Travis	2.4 km WNW of McNeil, Merril Cave	A	TNHC 21285–21287, 21634, 26795, 26796
10	Travis	Austin, Upper Bull Creek Park	A	TNHC 67474–67478
11	Travis	Austin, 3-Holer Cave (18)	A	TNHC 64213*
12	Travis	Austin, 2609 Westover Road (16)	B	TNHC 64147, 64149*
13	Travis	Austin, south shore of Colorado River at Red Bud Isle (14)	B	TNHC 64158
14	Travis	Austin, Brackenridge Field Laboratory (15)	B	TNHC 64151*, 64168
15	Travis	8 km SW of Austin on Barton Creek, Gaines Ranch	B	TNHC 1830, 5853, 5858, 5861, 5862, 5864, 5865, 53917, 53918, 53921, 53922, 53929, 53939, 53941, 53942, 53949
16	Hays	Fern Bank Spring (11)	C	TNHC 6040, 9440, 64202*, 64204, 64206, 64208, 64209
17	Hays	Rattlesnake Cave (10)	C	TNHC 61366, 61367, 61369, 61371–61375, 61382*, 61383
18	Hays	Wonder World Cave (9)	C	TNHC 64196, 64197*, 64198, 64200
19	Hays	Ezell's Cave	C	TNHC 6041
20	Comal	Washington Cave (7)	E	TNHC 62459*
21	Kendall	Grand Column Cave (6)	E	TNHC 62454*, 62456
22	Kendall	18 km E of Boerne, Cave Without a Name, and vicinity	E	TNHC 88, 89, 9445
23	Kendall	19.3 km NNE of Boerne, Dead Man's Cave	E	TCWC 78852, TNHC 9446, 21206–21209
24	Kendall	0.6 km W of Century Caverns, Able's Grotto	E	TCWC 38858–38860
25	Kendall	Spring Creek Cave	E	TNHC 64236
26	Kendall	8 km SE of Boerne, Grosser's Sink	E	TNHC (TTU) R7474
27	Kerr	Cherry Creek Ranch, entrance to Antonio's Cave (5)	E	TNHC 64179*
28	Kerr	10 km SW of Hunt on S side of TX Hwy 39 (4)	D	TNHC 63853, 63854*
29	Kerr	Mangus Swallow Cave	D	TNHC (TTU) 4384, 4385, 4390
30	Kerr	20.8 km W of Hunt on FM 1340, Kerr Wildlife Management Area	D	TNHC 46453
31	Bandera	Lost Maples State Natural Area	D	TCWC 80082, 80859, UTACV 24859, 24861, 24862, 24864, 64866, 64869
32	Bandera	6.4 km N and 0.8 km W of Vanderpool	D	TCWC 67874–67876
33	Real	3.2 km N of Leakey, cave on Bonner Ranch	D	TNHC 21250, 21251
34	Real	FM 337, outside Blue Oak Ranch (2)	D	TNHC 63849*
35	Edwards	20.6 km S of TX Hwy 41 on FM 335 (1)	D	TNHC 63679*, 63680*

width, head depth, snout length, and interorbital distance) and tail (tail width, tail height) characters with sex and group as factors and logSVL as the covariate.

Head width was found to be a particularly important character that varied among groups. To assess which populations differed significantly in head width, we conducted ANOVAs separately for each sex using the residual values from the regression of head width on SVL. Tukey tests were used to identify those populations with significant differences.

Multivariate analyses were then used to assess whether the groups can be differentiated morphologically. We conducted a principal components analysis (PCA, based on a correlation matrix) on the 12 morphometric characters to isolate body size to a single factor, principal component (PC) I. We selected the minimum number of components that explained at least 90% of the observed variance (components I–V) for further analyses. As was done for the individual traits, each principal component was analyzed using an ANOVA with sex and group as factors.

We explicitly tested the null hypotheses that the five mtDNA-defined groups do not differ in body shape and size, and body shape alone. Differences among groups in body shape and size were tested with a MANOVA using PC I–V as the dependent variables with sex and the mtDNA-defined groups as factors. Differences in body shape were tested two different ways: first with an additional MANOVA using only

Table 2. Mean ± 1 SD for 12 Morphological Traits. Within each row, the top values are for the uncorrected measurements (mm) and the bottom values are for the residuals from regressions of log<trait> on logSVL.

	Group A		Group B		Group C	
Trait	Male (n = 12)	Female (n = 11)	Male (n = 11)	Female (n = 10)	Male (n = 7)	Female (n = 15)
SVL	61.76 ± 5.48	59.80 ± 4.42	54.64 ± 2.92	53.25 ± 4.15	61.34 ± 5.22	58.23 ± 6.42
Head length	14.67 ± 1.14	13.67 ± 1.23	12.98 ± 0.56	12.25 ± 0.98	13.87 ± 0.49	13.25 ± 1.40
	0.009 ± .005	−0.010 ± 0.023	0.003 ± 0.011	−0.012 ± 0.018	0.001 ± 0.013	−0.010 ± 0.019
Head width	8.36 ± 0.84	8.02 ± 0.58	7.37 ± 0.50	7.29 ± 0.60	8.65 ± 0.56	8.17 ± 0.91
	−0.010 ± 0.021	−0.014 ± 0.018	−0.016 ± 0.019	−0.011 ± 0.015	0.009 ± 0.016	0.004 ± 0.014
Head depth	4.0 ± 0.59	3.97 ± 0.37	3.47 ± 0.24	3.37 ± 0.19	3.92 ± 0.32	3.65 ± 0.42
	0.001 ± 0.032	0.012 ± 0.046	−0.010 ±0.034	0.011 ± 0.028	−0.002 ± 0.015	0.031 ± 0.019
Snout length	2.94 ± 0.41	2.68 ± 0.26	2.52 ± 0.18	2.45 ± 0.22	2.86 ± 0.40	2.73 ± 0.39
	0.006 ± 0.033	−0.019 ± 0.015	−0.001 ± 0.030	−0.003 ± 0.036	−0.002 ± 0.039	0.002 ± 0.036
Interorbital distance	4.53 ± 0.61	4.35 ± 0.35	3.90 ± 0.35	3.81 ± 0.35	4.54 ± 0.27	4.25 ± 0.50
	0.004 ± 0.034	0.002 ± 0.024	−0.009 ± 0.030	−0.008 ± 0.021	0.011 ± 0.025	0.003 ± 0.041
Orbit width	2.60 ± 0.14	2.42 ± 0.25	2.34 ± 0.19	2.26 ± 0.18	2.54 ± 0.28	2.56 ± 0.30
	−0.001 ± 0.028	−0.024 ± 0.038	−0.010 ± 0.032	−0.017 ± 0.017	−0.010 ± 0.045	0.009 ± 0.032
Forelimb length	13.37 ± 1.0	13.24 ± 1.23	12.45 ± 0.63	11.95 ± 0.98	13.85 ± 0.67	13.16 ± 1.01
	−0.009 ± 0.020	−0.004 ± 0.026	−0.002 ± 0.025	−0.012 ± 0.014	0.009 ± 0.014	0.003 ± 0.019
Pectoral width	6.47 ± 0.77	6.42 ± 0.57	5.98 ± 0.64	5.96 ± 0.59	6.70 ± 0.50	6.45 ± 1.02
	−0.014 ± 0.029	−0.005 ± 0.029	−0.004 ± 0.048	0.006 ± 0.042	0.005 ± 0.028	0.005 ± 0.033
Hind limb length	15.21 ± 1.35	14.63 ± 1.27	14.08 ± 0.51	13.85 ± 0.86	15.06 ± 0.94	14.82 ± 1.33
	−0.003 ± 0.023	−0.010 ± 0.024	−0.001 ± 0.017	−0.001 ± 0.019	−0.005 ± 0.017	0.003 ± 0.023
Tail width	4.30 ± 0.57	4.31 ± 0.34	3.71 ± 0.50	3.94 ± 0.71	4.46 ± 0.35	4.22 ± 0.73
	−0.013 ± 0.038	0.004 ± 0.035	−0.027 ± 0.051	0.012 ± 0.067	0.008 ± 0.026	0.002 ± 0.037
Tail height	4.27 ± 0.65	4.33 ± 0.46	3.77 ± 0.73	4.21 ± 0.95	4.83 ± 0.39	4.47 ± 0.72
	−0.033 ± 0.050	−0.011 ± 0.055	−0.044 ± 0.080	0.016 ± 0.095	0.027 ± 0.032	0.010 ± 0.042

PC II–V and second with a MANCOVA using the 11 morphometric characters with logSVL as the covariate and sex and group as factors. All aforementioned statistical analyses were conducted in Systat 11 (Systat Software, Inc., Chicago, IL).

We also wanted to conduct cluster analyses to examine variation among the five mtDNA-defined groups. A discriminant function analysis would be ideal for this, but this technique needs larger per-group sample sizes than were available for this study. Thus, we calculated the Euclidean distances among group means and then used a modification of Collyer and Adams (2007) to calculate a *P*-value by randomizing group membership. For each pair of groups, we calculated the Euclidean distance between their means. We then randomized group membership within each pair of populations being examined and recalculated the distance between the means of the new groups. This was repeated for 1000 randomizations. The *P*-value was calculated as the proportion of times that the distance between the group means was greater in the randomizations than in the observed groups. This analysis was conducted for all variables and also for PC II–V in R, version 2.11.1 (R Development Core Team, 2010).

RESULTS

Summary statistics for the 12 morphometric characters are listed in Table 2. Seven traits were found to differ significantly among groups, and four of these—head width, snout length, interorbital distance, and orbit width—are head characters (Table 3, Fig. 2). Group B individuals are much smaller than individuals from the other groups (Table 2, Fig. 2D). Additionally, the significant among-group differences for head width, interorbital distance, and orbit width are driven largely by the relatively greater values found in group D (Tables 2, 3, Fig. 2A–C). Similarly, snout length and forelimb length are greater in group E (Tables 2, 3).

Head width was the trait with the greatest significance value in the trait-specific ANCOVAs ($P < 0.001$; Table 3). Tukey tests following sex-specific ANCOVAs of residual values were conducted to determine which groups were most different in relative head widths. For males, populations A and D ($P = 0.013$), populations B and D ($P = 0.002$), and populations B and E ($P = 0.028$) were significantly different. Similarly, for females, populations A and D ($P = 0.001$) and populations B and D ($P = 0.006$) were significantly different. These patterns are also suggested by Fig. 2A. Note that these results continue to highlight the unique morphology of group D.

Sexual dimorphism was detected in SVL, head length, and interorbital distance (Table 3, Fig. 2B, D). Males have greater SVL and proportionately longer heads in all groups (Table 2). Male-biased dimorphism in interorbital distance was largely restricted to groups C, D, and E, with the greatest dimorphism in E (Table 2, Fig. 2B). A MANCOVA on the cranial characters revealed sexual shape dimorphism (Wilks' Lambda = 0.788, $F_{5,91} = 4.89$, $P < 0.001$) with males having more massive heads (Table 2, Fig. 2F). The univariate ANCOVAs demonstrated that this result was largely driven by head length and interorbital distance (Table 3), although head width and snout length also tended to be greater in males (Table 2). Sexual shape dimorphism was not recovered for the tail characters (Wilks' Lambda = 0.976, $F_{2,94} = 1.165$, $P = 0.316$). For both cranial and tail characters, sexual shape dimorphism did not differ among populations (i.e., the interaction term was not significant. Cranial characters:

Table 2. Continued.

	Group D		Group E	
Trait	Male ($n = 6$)	Female ($n = 16$)	Male ($n = 11$)	Female ($n = 7$)
SVL	60.51 ± 5.16	53.30 ± 7.51	60.84 ± 6.67	56.01 ± 8.11
Head length	14.42 ± 1.41	12.67 ± 1.48	14.53 ± 1.62	13.23 ± 7.72
	0.008 ± 0.010	0.003 ± 0.011	0.010 ± 0.019	0.002 ± 0.017
Head width	8.84 ± 0.83	7.71 ± 0.96	8.61 ± 1.01	7.72 ± 1.02
	0.023 ± 0.019	0.014 ± 0.014	0.009 ± 0.019	−0.005 ± 0.028
Head depth	4.19 ± 0.42	3.45 ± 0.52	4.05 ± 0.49	3.57 ± 0.25
	0.031 ± 0.019	−0.002 ± 0.021	0.014 ± 0.038	−0.004 ± 0.041
Snout length	2.84 ± 0.43	2.38 ± 0.38	2.98 ± 0.37	2.76 ± 0.32
	−0.001 ± 0.042	−0.016 ± 0.039	0.020 ± 0.023	0.027 ± 0.033
Interorbital distance	4.60 ± 0.60	3.94 ± 0.51	4.42 ± 0.51	3.71 ± 0.60
	0.020 ± 0.037	0.008 ± 0.040	0.002 ± 0.025	−0.041 ± 0.027
Orbit width	2.75 ± 0.26	2.43 ± 0.28	2.67 ± 0.30	2.40 ± 0.29
	0.028 ± 0.029	0.014 ± 0.029	0.014 ± 0.031	−0.007 ±0.031
Forelimb length	13.66 ± 1.02	12.05 ± 1.21	14.08 ± 1.40	13.04 ± 1.25
	0.007 ± 0.016	−0.008 ± 0.025	0.018 ± 0.025	0.011 ± 0.016
Pectoral width	6.40 ± 0.59	6.08 ± 0.82	6.56 ± 1.00	6.12 ± 0.89
	−0.010 ± 0.026	0.015 ± 0.033	−0.004 ± 0.037	−0.002 ± 0.031
Hind limb length	15.09 ± 1.10	13.79 ± 1.43	15.67 ± 1.28	14.4.5 ± 1.62
	−0.001 ± 0.021	−0.002 ± 0.022	0.015 ± 0.016	0.003 ± 0.023
Tail width	4.39 ± 0.35	4.03 ± 0.72	4.29 ± 0.70	3.88 ± 0.74
	0.007 ± 0.030	0.021 ± 0.049	−0.008 ± 0.041	−0.014 ± 0.028
Tail height	4.84 ± 0.63	4.36 ± 0.80	4.37 ± 0.72	4.20 ± 1.24
	0.031 ± 0.057	0.033 ± 0.050	−0.017 ± 0.048	−0.006 ± 0.060

Wilks' Lambda = 0.830, $F_{20,302} = 0.878$, $P = 0.616$. Tail characters: Wilks' Lambda = 0.932, $F_{8,188} = 0.840$, $P = 0.568$).

The first five principal components explained 92% of the variance (Table 4). A plot of PCI versus PC II is shown in Figure 3. Patterns of variation in the principal components were consistent with the patterns in the original variables. Principal component I, which reflects overall body size, was sexually dimorphic and varies significantly among groups (Table 3, Figs. 2D, 3C). As with SVL (Table 2), sexual dimorphisms in PC I were greatest in groups C, D, and E (Fig. 3C). Principal component II, which reflects tail shape (heaviest loadings on tail height and width; Table 4), and PC IV, which reflects head shape (heaviest loadings on interorbital distance and head depth; Table 4), were also dimorphic (Table 3, Fig. 3E, F). Principal component IV (head shape) also differed among groups and between the sexes (Table 3, Fig. 3F).

Table 3. Sex, mtDNA Group, and Sex*Group Interactions for 12 Morphometric Characters and the First Five Principal Components. *P* values are from ANOVAs (SVL) or ANCOVAs (all other traits) with sex and group as factors and logSVL as a covariate. $P \leq 0.05$ are shown in bold.

log<Trait>	*n*	Sexes	Group	Sex*Group
SVL (ANOVA)	106	**0.002**	**0.002**	0.472
Head length	100	**<0.001**	0.102	0.756
Head width	106	0.150	**<0.001**	0.567
Head depth	106	0.059	0.066	0.171
Snout length	105	0.279	**0.039**	0.493
Interorbital distance	106	**0.039**	**0.019**	0.243
Orbit width	106	0.156	**0.006**	0.221
Forelimb length	106	0.080	**0.007**	0.591
Pectoral width	106	0.115	0.562	0.784
Hind limb length	105	0.600	0.267	0.704
Tail width	103	0.148	0.417	0.490
Tail height	103	0.140	**0.020**	0.386
PC I (body size)	106	**0.001**	**0.001**	0.296
PC II (tail shape)	106	**0.014**	0.093	0.377
PC III (orbit shape)	106	0.434	0.070	0.553
PC IV (head shape)	106	**0.020**	**0.002**	0.443
PC V (head shape2)	106	0.557	0.150	0.528

Multivariate analyses strongly rejected the null hypotheses that there are no differences in body shape and size or in body shape alone among the five groups. The groups were significantly different in body shape and size based on a MANOVA of PCs I–V (Wilks' Lambda = 0.530, $F_{20,306} = 3.232$, $P < 0.001$). Using body shape information alone (PC II–V), the groups were also strongly differentiated (Wilks' Lambda = 0.653, $F_{16,284} = 2.667$, $P = 0.001$). In a second test of shape-based differences, we used SVL as the covariate in a MANCOVA with the remaining 11 traits. This also yielded a strongly significant result (Wilks' Lambda = 0.330, $F_{44,327} = 2.496$, $P < 0.001$).

Euclidean distances among group means when examining all traits were strongly influenced by body size with group B found to be the most distinct (Table 5). When body size was excluded by examining PC II–V, significant differences were found in males between groups D and E ($P = 0.002$) and between A and D ($P = 0.355$), with nearly significant differences between C and E ($P = 0.058$) and A and E ($P = 0.068$). In females, only the comparison between groups C and E was found to be nearly significant ($P = 0.076$).

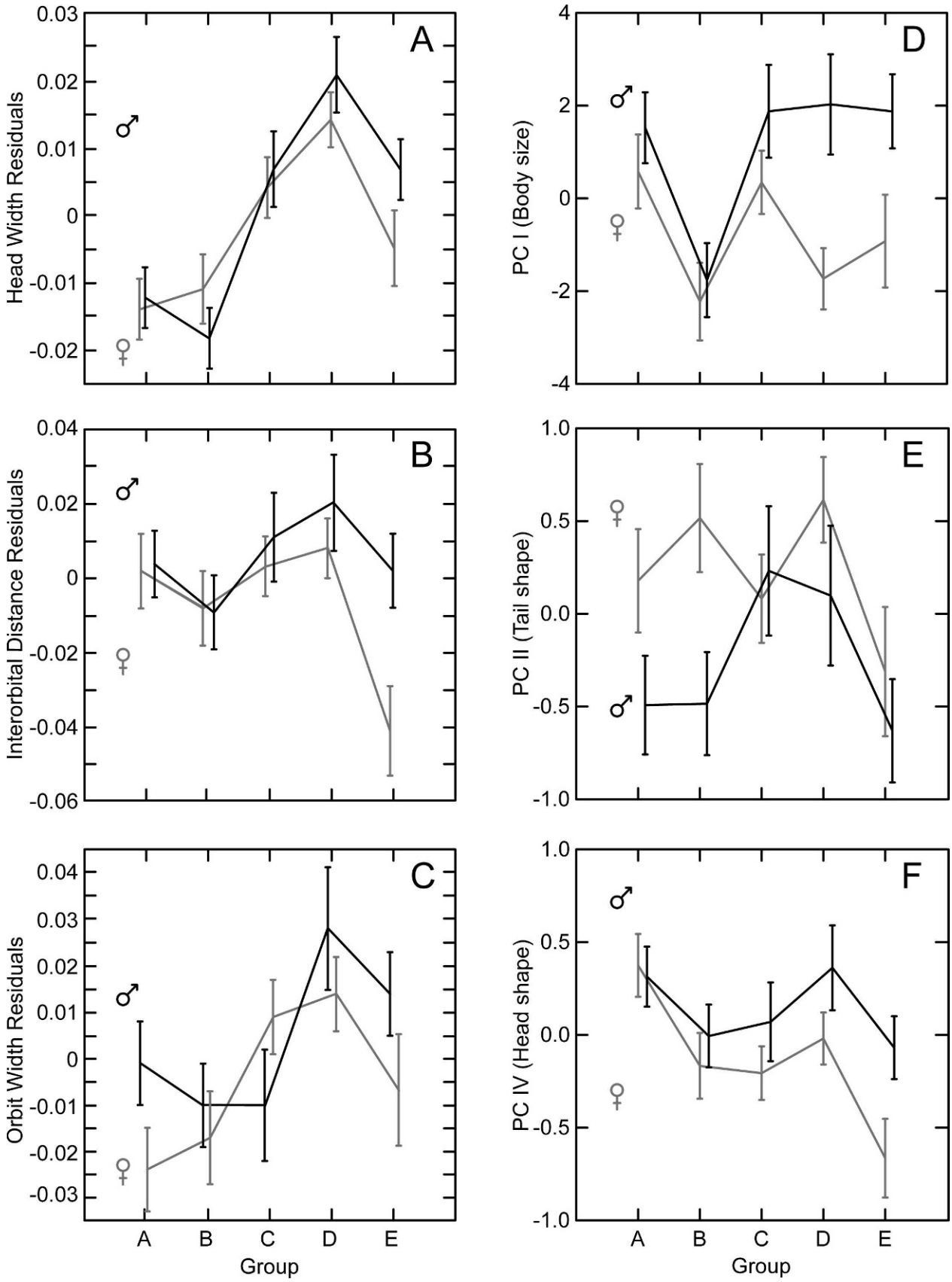

Fig. 2. Plots of mean ± 1 SD for three head characters (left column) and mean ± 1 SE for three principal components across the five mtDNA-defined groups. Dark lines are the males and the lighter gray lines are females.

Table 4. Principal Component Loadings for the First Five Components.

% Variance explained	PC I 73.9%	PC II 8.1%	PC III 4.2%	PC IV 3.0%	PC V 2.8%
SVL	0.961	−0.123	0.004	0.047	0.012
Head length	0.916	−0.280	−0.042	0.078	0.005
Head width	0.950	0.016	−0.013	0.035	0.021
Head depth	0.855	0.083	0.025	0.240	0.400
Snout length	0.861	−0.214	0.160	−0.161	0.238
Interorbital distance	0.851	0.013	0.016	0.399	−0.272
Orbit width	0.743	−0.147	−0.633	−0.092	−0.010
Forelimb length	0.874	−0.251	0.178	−0.144	−0.111
Pectoral width	0.840	0.220	−0.062	−0.198	−0.071
Hind limb length	0.864	−0.284	0.201	−0.132	−0.150
Tail width	0.844	0.472	0.062	0.026	−0.069
Tail height	0.726	0.622	0.022	−0.143	0.000

DISCUSSION

Morphological variability and cryptic lineages.—Plethodontid salamanders, and especially the *P. glutinosus* group, have long been recognized for their extreme morphological stasis (Wake et al., 1983; Larson, 1984, 1989; Carr, 1996). Nevertheless, we recovered morphological differences among the five mitochondrially defined groups of central Texas *P. albagula* despite these groups inhabiting a relatively small geographic area. These differences are evident in the MANOVA and MANCOVA results and in examination of variation in some of the individual traits (Fig. 2, Tables 2, 3). However, examination of the plot of PC I against PC II (Fig. 3) and the analyses of Euclidean distances among group means (Table 5) highlights that many groups were not dramatically distinct. Nevertheless, group B individuals are noticeably smaller than individuals from the adjacent groups A, C, and E (Table 5, Figs. 2D, 3). Group D is also quite distinct in several head characters (Tables 2, 3, Fig. 2), and group D males were easily differentiated from males in the adjacent group E in the analysis of Euclidean distances using PC II–V. On the other hand, the neighboring groups C and E are not as easily distinguished. Thus, there are coincident morphological and mitochondrial breaks between some groups suggesting that these mtDNA breaks result from actual disjunctions in gene flow (*sensu* Irwin, 2002). These results support the possibility of cryptic lineages of *P. albagula* within central Texas. Future studies using multiple nuclear DNA markers to examine gene flow in the contact zones between groups would be ideal for assessing whether groups are reproductively isolated and for further assessing the possibility of cryptic species within central Texas slimy salamanders.

Our morphological results echo those of Carr (1996), who was able to morphologically differentiate among a number of lineages in the *P. glutinosus* complex that were proposed to be species based on allozyme data. These two studies suggest that despite the generally accepted view of morphological conservatism in *Plethodon,* morphometric variation can still be used as one line of evidence to assess taxonomic boundaries in these salamanders.

Examination of the original characters indicates the morphological distinctiveness of lineage D particularly for head width, interorbital distance, and orbit width (Table 2, Fig. 2A–C). This lineage occurs in the driest habitat for *P. albagula* at the western edge of the species' range (Fig. 1). This may explain the generally more robust build and lower surface area to volume ratio of lineage D individuals (Table 2; head width, tail width, and tail height all suggest increased robustness of group D individuals).

Unexpected levels of morphological and molecular diversity in the Edwards Plateau is not unique to *P. albagula*. Morphological and molecular analyses of the nontransforming hemidactyliine salamanders (*Eurycea*) that inhabit the springs and caves of the Edwards Aquifer have also uncovered numerous cryptic lineages (Chippindale et al., 2000). Both *Plethodon* and *Eurycea* are endemic to the karst habitats of the Edwards Plateau, although *P. albagula* is a terrestrial species whereas the central Texas hemidactyliines are aquatic.

Previous mitochondrial DNA sequencing studies indicate that the *P. albagula* of the Edwards Plateau are a clade distinct from the geographically disjunct populations in the Interior Highlands (Baird et al., 2006; Kozak et al., 2006; Wiens et al., 2006). Here and in Baird et al. (2006), morphological and mtDNA sequence variation among the Edwards Plateau *P. albagula* has been examined, but there is a lack of morphological and/or molecular studies examining variation within the Interior Highlands populations of *P. albagula*. The available data are drawn from studies regarding the entire genus or the large *P. glutinosus* species group (Carr, 1996; Kozak et al., 2006; Wiens et al., 2006), all of which only have a small sample of *P. albagula*. This small sample nevertheless indicates large morphological differences among salamanders of these regions (Carr, 1996), and that taxonomic boundaries in Interior Highlands slimy salamanders are poorly understood (e.g., the finding of the Interior Highlands *P. albagula* being paraphyletic with respect to *P. sequoyah*; Kozak et al., 2006; Wiens et al., 2006). Given the differentiation observed here among the approximately 425 km long corridor of *P. albagula* on the Edwards Plateau, it is very probable that there are even greater molecular and morphological differences between the Edwards Plateau and Interior Highlands *P. albagula*. Such a finding would support the suggestion of Frost and Hillis (1990) that *P. albagula* (*sensu* Highton et al., 1989) includes multiple distinct species.

Sexual dimorphism.—The apparent male-biased sexual size dimorphism (i.e., males larger than females) in *P. albagula*

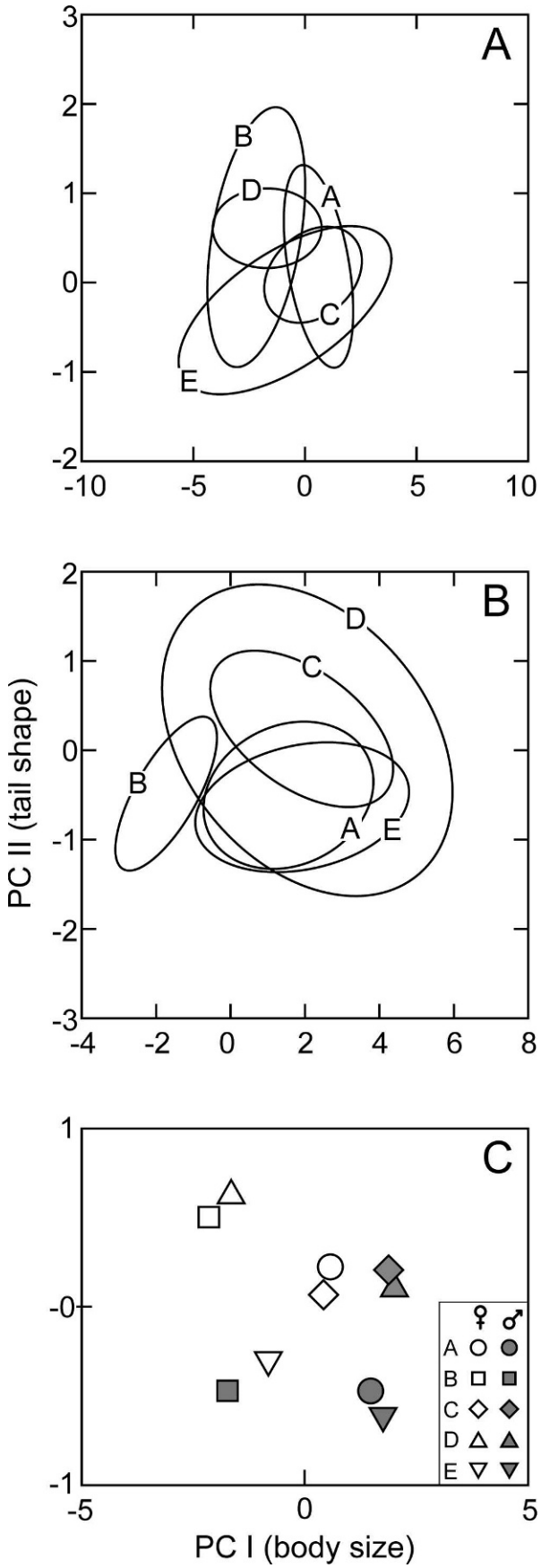

Fig. 3. Plots of PC I versus PC II. The 95% confidence ellipses are shown for females (A) and males (B). The group means (C) are also plotted for both sexes.

(Tables 2, 3, Fig. 3D) is counter to the sexual size dimorphism of other members of this genus. In all species of *Plethodon* in which mean SVL has been examined for both sexes, females are larger than males, except in *P. websteri* in which males and females are of equivalent size (Bruce, 2000). Within the *P. glutinosus* species group, the six species previously examined (*P. glutinosus, P. kentucki, P. metcalfi, P. ouachitae, P. teyahalee,* and *P. yonahlosee*) exhibit female-biased SSD, with females having larger mean SVL and reaching larger maximum SVL (Bruce, 2000). Similarly, three species in the *P. cinereus* group (*P. cinereus, P. hoffmani,* and *P. serratus*) and the three examined species in the Western Plethodon group (*P. larselli, P. neomexicanus,* and *P. vehiculum*) also show greater mean female size (Bruce, 2000).

Within North American plethodontids, male-biased SSD has been reported in *Desmognathus ochrophaeus* (Houck, 1988; Bruce, 1993). This dimorphism was attributed to larger males being able to secure access to females in male–male competition (Houck, 1988). However, in other species of plethodontids (e.g., *P. cinereus*) in which male–male competition is known and there is a large-male advantage, females remain the larger sex (Mathis, 1991; Bruce, 2000). These studies and the observed male-biased size dimorphism in central Texas *P. albagula,* which is most pronounced in groups C, D, and E (Table 2, Fig. 3D), suggest that there may be aspects to the reproductive biology of *P. albagula* that differ dramatically from those observed in close congeners of *P. albagula*. Alternatively, sex-specific ecological differences, such as dietary resource partitioning, could also explain the observed dimorphism.

Sexual shape dimorphism was recovered in the cranial characters, with males having more massive heads. Previous studies of other *Plethodon* have demonstrated that changes in head shape are associated with changes in prey size, with individuals with wider heads eating prey of larger size (Adams, 2000; Maerz et al., 2006). Thus, males may be consuming larger prey items than females. It is not clear, however, whether such resource partitioning, if it exists, is driving the difference in head morphology or is merely an artifact of this difference. For example, the larger head size could be associated with male–male competition with pleiotropic effects on prey consumption.

Head morphometrics and trophic morphology.—One unexpected result was the dramatic among-group differences in head width, and to a lesser degree, orbit width (Tables 2, 3). Head width also had the lowest *P*-value in the trait-specific ANOVAs ($P < 0.001$), and orbit width had the second lowest *P*-value in the trait-specific ANOVAs ($P = 0.006$; Table 3). These low *P*-values are largely driven by the uniqueness of group D individuals, which have relatively wider heads and eye orbits than the other groups (Table 2, Fig. 3A, C). It is not immediately clear what, if any, selective pressures would lead to these differences. It may be that these differences reflect the generally more robust build of group D salamanders, which may be associated with the higher aridity in this portion of the species range. Alternatively, the observed increase in head width in group D, suggests that these salamanders are eating larger prey than other Edwards Plateau *P. albagula* (Adams, 2000; Maerz et al., 2006). Thus, differences in prey availability across the Edwards Plateau could be impacting head morphology. A third possibility is that habitat differences among groups may be driving these morphological differences. Central

Table 5. Pairwise Euclidean Distances among Group Means. Values above the diagonal are the pairwise Euclidean distances among the means of each of the five mitochondrially defined groups. Values below the diagonal are the *P*-values for these comparisons. $P \leq 0.05$ are shown in bold. Values in each cell are given for males and then females.

	A	B	C	D	E
A	—	0.1817, 0.1518	0.0694, 0.0525	0.0743, 0.1344	0.0355, 0.1123
B	**0.002, 0.012**	—	0.2168, 0.1356	0.2266, 0.0479	0.1967, 0.0876
C	0.425, 0.544	**0.001, 0.046**	—	0.0512, 0.1131	0.0657, 0.0929
D	0.445, **0.046**	**0.001,** 0.751	0.777, 0.079	—	0.0647, 0.0917
E	0.872, 0.124	**0.006,** 0.379	0.476, 0.295	0.604, 0.373	—

Texas *P. albagula* are associated with karst outcroppings and caves of the Edwards Plateau; surface activity is confined to a small portion of the year when conditions are cool and wet. The remainder of the year is spent in subterranean habitats. Thus, the unique group D head width may reflect differences in subterranean habitat use or burrowing activity. Studies of prey and habitat use will help to discriminate among these hypotheses.

ACKNOWLEDGMENTS

We thank D. Hillis for advice on central Texas *Plethodon* and M. Benard, D. Bolnick, and S. Scarpino for statistical advice. For the loan of specimens, we thank T. LaDuc (Texas Natural History Collections, UT Austin), T. Hibbitts (Texas Cooperative Wildlife Collections, Texas A&M), and J. Campbell and C. Franklin (Amphibian and Reptile Diversity Research Center, UT Arlington). D. Cannatella, D. Hillis, T. LaDuc, and members of the Cannatella lab provided helpful comments on the manuscript.

LITERATURE CITED

Adams, D. C. 2000. Divergence of trophic morphology and resource use among populations of *Plethodon cinereus* and *P. hoffmani* in Pennsylvania: a possible case of character displacement, p. 383–394. *In*: The Biology of Plethodontid Salamanders. R. C. Bruce, R. G. Jaeger, and L. D. Houck (eds.). Kluwer Academic/Plenum Publishers, New York.

Adams, D. C., C. M. Berns, K. H. Kozak, and J. J. Wiens. 2009. Are rates of species diversification correlated with rates of morphological evolution? Proceedings of the Royal Society of London B 276:2729–2738.

Baird, A. B., J. K. Krejca, J. R. Reddell, C. E. Peden, M. J. Mahoney, and D. M. Hillis. 2006. Phylogeographic structure and color pattern variation among populations of *Plethodon albagula* on the Edwards Plateau of central Texas. Copeia 2006:760–768.

Bruce, R. C. 1993. Sexual size dimorphism in desmognathine salamanders. Copeia 1993:313–318.

Bruce, R. C. 2000. Sexual size dimorphism in the Plethodontidae, p. 243–260. *In*: The Biology of Plethodontid Salamanders. R. C. Bruce, R. G. Jaeger, and L. D. Houck (eds.). Kluwer Academic/Plenum Publishers, New York.

Carr, D. E. 1996. Morphological variation among species and populations of salamanders in the *Plethodon glutinosus* complex. Herpetologica 52:56–65.

Chippindale, P. T., A. H. Price, J. J. Wiens, and D. M. Hillis. 2000. Phylogenetic relationships and systematic revision of central Texas hemidactyliine plethodontid salamanders. Herpetological Monographs 14:1–80.

Collyer, M. L., and D. C. Adams. 2007. Analysis of two-state multivariate phenotypic change in ecological studies. Ecology 88:683–692.

Conant, R., and J. T. Collins. 1998. A Field Guide to Reptiles and Amphibians: Eastern and Central North America. Houghton-Mifflin Company, Boston.

Frost, D. R., and D. M. Hillis. 1990. Species in concept and practice: herpetological applications. Herpetologica 46: 87–104.

Grobman, A. B. 1944. The distribution of the salamanders of the genus *Plethodon* in eastern United States and Canada. Annals of the New York Academy of Sciences 45:261–316.

Highton, R. G., G. C. Maha, and L. R. Maxson. 1989. Biochemical evolution in the slimy salamanders of the *Plethodon glutinosus* complex in the eastern United States. Illinois Biological Monographs 57:1–153.

Houck, L. D. 1988. The effect of body size on male courtship success in a plethodontid salamander. Animal Behavior 36:837–842.

Irschick, D. J., and H. B. Shaffer. 1997. The polytypic species revisited: morphological differentiation among tiger salamanders (*Ambystoma tigrinum*) (Amphibia: Caudata). Herpetologica 53:30–49.

Irwin, D. E. 2002. Phylogeographic breaks without geographic barriers to gene flow. Evolution 56:2383–2394.

Kozak, K. H., D. W. Weisrock, and A. Larson. 2006. Rapid lineage accumulation in a non-adaptive radiation: phylogenetic analysis of diversification rates in eastern North American woodland salamanders (Plethodontidae: *Plethodon*). Proceedings of the Royal Society of London B 273:539–546.

Larson, A. 1984. Neontological inferences of evolutionary pattern and process in the salamander family Plethodontidae. Evolutionary Biology 17:119–217.

Larson, A. 1989. The relationship between speciation and morphological evolution, p. 579–598. *In*: Speciation and Its Consequences. D. Otte and J. A. Endler (eds.). Sinauer Associates, Sunderland, Massachusetts.

Maerz, J. C., E. M. Myers, and D. C. Adams. 2006. Trophic polymorphism in a terrestrial salamander. Evolutionary Ecology Research 8:23–35.

Mathis, A. 1991. Large male advantage for access to females: evidence of male–male competition and female discrimination in a territorial salamander. Behavioral Ecology and Sociobiology 29:133–138.

Pauly, G. B., O. Piskurek, and H. B. Shaffer. 2007. Phylogeographic concordance in the southeastern United States: the flatwoods salamander, *Ambystoma cingulatum*, as a test case. Molecular Ecology 16:415–429.

R Development Core Team. 2010. R: A language and environment for statistical computing, version 2.11.1. R Foundation for Statistical Computing, Vienna. http://cran.r-project.org

Ryan, T. J., and R. C. Bruce. 2000. Life history evolution and adaptive radiation of hemidactyliine salamanders p. 303–326. *In*: The Biology of Plethodontid Salamanders. R. C. Bruce, R. G. Jaeger, and L. D. Houck (eds.). Kluwer Academic/Plenum Publishers, New York.

Wake, D. B., G. Roth, and M. H. Wake. 1983. On the problem of stasis in organismal evolution. Journal of Theoretical Biology 101:211–224.

Wiens, J. J., T. N. Engstrom, and P. T. Chippindale. 2006. Rapid diversification, incomplete isolation, and the ''speciation clock'' in North American salamanders (genus: *Plethodon*): testing the hybrid swarm hypothesis of rapid radiation. Evolution 60:2585–2603.