VARIABILITY IN SURVIVAL, GROWTH AND METAMORPHOSIS IN THE LARVAL FIRE SALAMANDER (*SALAMANDRA SALAMANDRA*): EFFECTS OF LARVAL BIRTH SIZE, SIBSHIP AND ENVIRONMENT

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ABSTRACT: *Salamandra salamandra* has an unusual mode of reproduction. Females retain eggs in their oviducts and give birth to aquatic larvae or, in some populations, to fully metamorphosed juveniles. We investigated how variation in the birth size of larvae of five different sibships of *S. salamandra* affected larval survival, larval growth rates, time to metamorphosis, and size at metamorphosis under different food and temperature conditions. Maternal effects, via the effect of larval birth size, attenuated throughout development in all environments but still affected metamorphic traits at lower temperatures. Larval developmental time was negatively correlated with larval birth size and positively correlated with size at metamorphosis. After controlling for the effect of larval birth size, broad-sense heritabilities were obtained for residual values of metamorphic traits, indicating that enough genetic variability exists at lower temperature environments and that responses to selection of metamorphic traits are possible. The size at and the time to metamorphosis were phenotypically and genetically correlated. Since both traits presumably affect fitness, genetic dependence between size and time to metamorphosis may act as a constraint on adaptive evolution through antagonistic pleiotropy. Mass-specific growth, time to metamorphosis, and size at metamorphosis were plastic under different temperature and food conditions, but no significant sibship $\times$ environment interaction was found for any trait. In addition, significant cross-environment correlation for size at metamorphosis with respect to the food gradient indicates low potential for the adaptive evolution of reaction norms to heterogeneous food environments.

Key words: Maternal effects; Metamorphosis; Plasticity; *Salamandra salamandra*; Sibship variation; Trade-offs

VARIATION in offspring traits that confer fitness upon which natural selection may act is due to inter-individual differences in genotype, environmental conditions (including maternal effects), and genotype $\times$ environment interactions (the variation in the relative expression of genotypes in different environments; Lewontin, 1974). Maternal effects are considered the source of individual phenotypic variation attributable not to an organism's own genotype or its interaction with the environment but to the phenotype or environment of its mother (Wade, 1998). Since maternal effects are the expression of the maternal phenotype and have an inherent genetic component, such traits should evolve in much the same way as any other trait that shows Mendelian inheritance. Maternal effects in species that provide no parental care to their offspring are basically limited to the investment the female may provide to the eggs. Usually, maternal effects have their maximal influence during the earlier

stages of ontogeny (a period of greatest mortality). Egg and propagule size variations have been empirically demonstrated to have strong effects on offspring fitness traits in plants and animals (Azevedo et al., 1997; Roach and Wulff, 1994). Moreover, maternal effects are variable in duration, and their expression is often contingent on the environment in which the offspring develops (Bernardo, 1996).

In amphibians that do not provide parental care, variation in egg size determines hatchling size and, therefore, size at the start of the free-feeding stage. Egg size may also correlate with traits that may confer fitness, such as faster developmental rates or higher growth rates (for a revision see Kaplan, 1998). In addition, the effect of egg size is modulated by environmental factors such as temperature and food availability (Berven and Chadra, 1988; Kaplan, 1992). Many amphibians occupy temporary ponds during their larval stage. These habitats are very unpredictable and highly variable with respect to their duration, temperature, food resources, and the density of individuals that

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occupy them (Newman, 1992). Organisms inhabiting these aquatic environments are under strong selection pressure through pond desiccation, predation, and competition (Alford, 1999). As a consequence of environmental heterogeneity, the ability of a given genotype to modulate its phenotype depending on the environment (known as phenotypic plasticity), may be adaptive if it is capable of developing optimal phenotypes in two or more environments (Via, 1987). The ability to evolve adaptive phenotypic plasticity is limited by: (1) a lack of genetic variability either in plasticity (genotype $\times$ environment variance) or within environments; (2) genetic correlations between character states across environments that limit the evolution of the reaction norms; and (3) genetic trade-offs with other characteristics that affect fitness (Via and Lande, 1985).

The fire salamander, *Salamandra salamandra*, which is widely distributed throughout Europe and the Middle East (Thorn and Raffaelli, 2001), has an unusual mode of reproduction. Females of most populations do not lay eggs but retain them in their oviducts until the egg yolk has been exhausted, giving birth to well developed aquatic larvae (ovoviparous populations). In addition, females from some populations of the northern Iberian Peninsula are able to retain their offspring throughout development and give birth to fully metamorphosed terrestrial juveniles (viviparous populations; Dopazo and Alberch, 1994; Joly, 1968). This long brooding effort by female fire salamanders probably increases the variance induced by maternal effects, which may affect larval performance and some metamorphic traits. We examined: (1) the effect of variation of larval size at birth, presumably a maternally induced trait, on survival, growth, and time to metamorphosis and the persistence of this effect throughout larval ontogeny under two environmental gradients of temperature and food availability; (2) the potential evolution of metamorphic traits by analyzing full-sibship variation that provides both broad-sense heritabilities for larval growth rate, size, and time to metamorphosis within environments (after controlling for larval birth size as a surrogate of maternal effects) and phenotypic and genetic correlations between metamorphic traits; and (3) phenotypic plasticity and its genetic variation for metamorphic traits.

MATERIAL AND METHODS

Animal Collection

Five gravid females were captured at night during the final week of December 1994 at Puerto de Mijares (Avila Province, central Spain, 40° 20' N, 05° 11' W). The population at Puerto de Mijares (*S. salamandra bejarae*; García-París et al., 2003) is an ovoviparous population, breeding in streams that usually dry up by mid-or late summer; although, in some years, water remains and allows larvae to metamorphose during autumn. Collected females gave birth in the laboratory a few weeks after their capture, during 10–20 January 1995.

Experimental Design

Recently born larvae were kept at one of two temperature treatments, 20–25 C (high temperature) or 15–18 C (low temperature) under a 12h:12h light-dark regime that was synchronized with the natural light cycle. Larvae were fed to near satiation, at either two food treatments: once a week (low food) or three times a week (high food) with frozen chironomid worms. This schedule defined four treatments: high temperature-high food (TF), high temperature-low food (Tf), low temperature-high food (tF), and low temperature-low food (tf). We randomly allocated 9–12 larvae from each female to each treatment, and each was raised individually in 0.3-l plastic containers filled with 250 ml of dechlorinated tap water, changed after each feeding. Within each temperature treatment, we randomly assigned containers on a bench, which yielded a two-way, completely randomized design. As the larvae approached metamorphosis (when individuals start to lose their gills and their tail fins become reduced), moss was added to the containers to prevent drowning. All containers were covered with a plastic lid to avoid escape by any specimen. The first metamorphs appeared at the beginning of June (TF treatment). The date of metamorphosis of each individual was recorded as the date of complete gill resorption. At this point, individuals were measured and weighed.

Response Variables and Data analysis

Larvae were weighed (g) and measured (total length) the day after they were born. These measurements were repeated every

2 wk during the larval phase to analyze growth patterns. Growth rates were also analyzed on a mass-specific basis, following the method of Sinervo and Adolph (1989). Growth in mass was expressed as:

$$\text{mass-specific growth rate} \\ = (\ln[\text{mass } t_2] - \ln[\text{mass } t_1]) / t_2 - t_1,$$

where t_1 and t_2 are the birth and metamorphosis dates, respectively. All response variables were $\log_e$ transformed to meet assumptions of homogeneity of variances and normality.

Survival was analyzed using the STATISTICA 5.5 Visual GLZ Statistical Package (Statsoft, Inc., 1999), fitting a binomial distribution of the data with a Logit Link function to yield maximum-likelihood ratio estimates. The analysis of parallelism of growth between larvae of different sibships was performed by multivariate repeated measures MANOVA using the module General ANOVA/MANOVA in STATISTICA 5.5 (Statsoft, Inc., 1999). We employed MANOVA to bypass the assumption of compound symmetry and sphericity (von Ende, 1993). A specific test was performed on the differences in the shape of the growth curves by examining the sibship $\times$ time interaction. A significant interaction implies sibship variation in growth rates over time; nonsignificance indicates that sibship growth trajectories run parallel to each another.

Analysis of the Genetic Variation of Growth and Metamorphic Traits

Heritability estimates of metamorphic traits were based on full-sib covariance of individual larvae. Full-sib covariance contains, in addition to epistatic effects, those effects due to dominance, nongenetic maternal effects, and common family environment, all confounded with estimates of the additive gene effects. They, thus, provide a broad sense estimate of heritability (H^2), which is the fraction of the total phenotypic variance attributable to both additive and nonadditive (including dominance and epistasis) genetic variances (Roff, 1997). The larval salamanders used in our study were presumed to represent sets of full siblings. However, multiple paternities cannot be discounted because females can store sperm over long periods (Thorn and Raffaelli,

2001). If multiple paternities did occur, this would lead to underestimations of heritabilities (Schwartz et al., 1989). Because salamanders were allocated randomly to plastic containers and raised individually, common environmental variance probably did not obscure the genetic differences between individuals. Nongenetic maternal effects contribute to the covariance of full siblings, thus increasing estimates of heritability (Willham, 1963). This effect, basically mediated through salamander birth size, greatly affected metamorphic variables (as shown below). Therefore, estimates of heritabilities and genetic covariances were made on the residuals of response variables of larval size at birth (Garland, 1994). One potential concern is whether larvae belonging to different females were in different stages of development at birth; if so, then we could confound the effect of size at birth with stage at birth. We can dismiss this possibility since salamanders give birth to larvae at the same stage of development, which can be determined by the pattern of skull ossification, which reflects the degree of cranial ossification at birth, and the process of remodelling of hyobranchial and palate (Dopazo, 1995).

Estimates of causal components of variance were obtained within each particular treatment using the nf3 program of the Quercus package (Shaw and Shaw, 1992), compiled in Pascal under VMS and run on an AXP 2100. The Quercus program estimates genetic and environmental components of phenotypic variance by a restricted maximum likelihood (REML) method based on Shaw (1987). In our full-sib design, which included no parental morphometric data, the program estimated broad-sense genetic and environmental components (i.e., the confounding variance due to additive, dominance, and maternal effects). To test the hypothesis that certain variance components were >0 , the log likelihood ($L_{\max}$) for the unconstrained estimates was compared to L_0 , the log likelihood of the estimates under the null hypothesis. The L_0 is obtained by constraining to zero the components for which the test is desired. If both the unconstrained (alternative model) and the constrained (null hypothesis model) analyses converge with feasible estimates, the asymptotic distribution of $2 \times (L_{\max} - L_0)$ has a Chi-squared distribution with v degrees of freedom (where v is the

number of additional components constrained in the null hypothesis model). Significantly large values for $2 \times (L_{\max} - L_0)$ means that the constrained model fits the data significantly better than the unconstrained model (Manly, 1985).

Phenotypic plasticity in metamorphic traits was analyzed between environments using two-way mixed model ANCOVA's with a composite environmental factor, a sibship factor, and larval mass at birth as a covariate. The composite environmental factor was the three combinations of food and temperature treatments that yielded metamorphs (TF, tF, tf). We used pairs of treatments to define two environmental gradients. The first gradient was variation in temperature (combining high and low temperature at high food—TF vs. tF), and the second was food variation (combining high and low food at low temperature—tF vs. tf). The sibship $\times$ environment interaction estimates the amount of variation among genotypes in response to differing food and temperature environments and, thus, estimates the genetic variability for this plasticity. The different food and temperature levels were considered "fixed effects," and sibships were treated as a "random effect." We used a mixed model ANCOVA with Type III expected mean squares method (PROC GLM in SAS; SAS Institute, Inc., 1990; Model II in Ayres and Thomas, 1990; the SAS model in Fry, 1992). Type III SS accounts for unequal cell size and produces orthogonal tests of hypotheses (SAS Institute Inc., 1990), which gives a conservative measure of treatment effects.

Genetic correlations were estimated within particular environments based on the REML that partitioned the covariation between measures of larval size at birth and the time to and size at metamorphosis. In addition, the covariation between adjusted time to and size at metamorphosis was estimated, after controlling for larval birth size. The genetic covariance (COV_G) was tested for statistical significance by comparing the likelihood of a model with the genetic covariance constrained to zero to that of the less-constrained model using 1 df. Additional estimates of genetic correlations were also made using the family mean method, r_m being the Pearson product-moment correlation between family trait means for both traits (Via, 1984). Phenotypic correlations were

obtained for each environment using Pearson product-moment correlations. Genetic correlations were also estimated across environments using the family mean method, r_m being the Pearson product-moment correlation between the family trait means expressed in both environments (Via, 1984). Adjusted size at metamorphosis and time to metamorphosis in different environments were considered distinct but potentially inter-correlated character states (Falconer, 1952).

RESULTS

The Effect of Larval Birth Size on Survival, Growth, and Metamorphosis

Larval mass at birth was 0.15 ± 0.08 g (range 0.08–0.23 g, $n = 209$). Most of this variation was explained by female identity (broad-sense heritability, including nongenetic maternal effects, $H^2 = 0.94$, $\chi^2 = 96.74$, $P < 0.00001$, $n = 209$). Coefficients of variation ranged between 8.19 for female #3 and 11.91 for female #2. The average size of larvae belonging to sibship #5 was significantly smaller than in other sibships (Tukey HSD test for unequal sample size; $P < 0.005$ in all post-hoc comparisons; see larval body mass at birth; Fig. 1).

Survival differed across experimental treatments. No survival to metamorphosis was observed for treatment Tf. Survival with treatment TF was 71.7%, while survival was 98.2% and 98.1% for tF and tf, respectively. Significant differences were found between TF and the two other treatments (test of difference between two percentages; $P < 0.001$ in both cases; (Brown and Hollander, 1977). For these two latter treatments, female identity explained variation in survival ($\chi^2 = 84.70$, $P < 0.0001$). In treatment TF, larval size at birth explained variation in survival ($\chi^2 = 5.07$, $P = 0.02$), with large larvae surviving better than small ones. However, in addition to and after controlling for larval birth size, female identity also explained variation in larval survival ($\chi^2 = 19.68$; $P < 0.0002$). The larvae of females #4 and #5 showed poorer survival than those of other families (Fig. 2). In the Tf treatment, no larvae reached metamorphosis, but larval time-to-death varied as a function of sibship identity ($F_{4,40} = 7.23$, $P = 0.0002$). Birth sizes covaried positively with time-to-death, ($r = 0.39$, $P = 0.01$, $n = 42$). After controlling for variation in

larval birth size, the female identity effect remained significant ($F_{4,39} = 4.89$, $P = 0.003$). The average time-to-death of the offspring of female #4 and #5 was shorter than those of others.

Initial larval size affected later growth. Larval mass at birth correlated with size at different larval ages for each particular treatment before the first larva reached metamorphosis (Table 1). A positive covariation was found between larval birth size and subsequent sizes under the tF and tf treatments throughout the larval period. However, the covariation between initial and subsequent size was independent after 6 wk of growth under the TF treatment.

Birth size correlated phenotypically and genetically with metamorphic traits and mass-specific growth rates (Table 2). With the tf treatment, size at metamorphosis covaried positively with birth size, both phenotypically and genetically (REML estimate). Time to metamorphosis correlated negatively at the phenotypic level in low temperature treatments. Finally, negative phenotypic correlations were found with mass-specific growth in all treatments. In addition, genetic covariance between both traits was found for the low temperature treatments.

Sibship Variation in Larval Growth and Metamorphic Traits

Estimates of broad-sense heritabilities of larval size at the age of 10 wk, based on the residuals of larval birth size, showed no sibship variation in mass-specific growth rates (Table 3). Growth profiles were obtained of the sibships in each environment from birth to the time the first metamorphs appeared (week 14 with the TF and tF treatments, and week 22 with the tf treatment; Fig. 1). Repeated measures MANOVA showed that the larvae grew constantly over time and that mean growth response differed with respect to sibship (Table 4). However, the interaction sibship $\times$ time, which tests the variation attributable to changes in growth rate among sibships, was not significant for any of the three treatments. Thus, larval mean growth rate did not change between sibships over time.

The variance in metamorphic traits in tf and tF treatments was largely explained by sibship, as revealed by the broad-sense heritability

FIG. 1.—Larval *Salamandra salamandra* mass (mean $\pm$ 1 SE) for the five different sibships in three growing environments from birth to the appearance of the first metamorph (week 14 for the high temperature-high food and low temperature-high food treatments, and week 22 for the low temperature-low food treatment). Treatments were: TF (high temperature-high food), tF (low temperature-high food), tf (low temperature-low food).

exhibited by date at metamorphosis and size at metamorphosis, after controlling for the effect of larval birth size. No genetic variation was found for salamanders metamorphosing under the TF treatment (Table 3). Mass-specific growth showed no genetic variability in any environment. Length of the larval period and size at metamorphosis were highly and positively correlated, both phenotypically and genetically, in the three experimental environments (Table 5; Fig. 3). A homogeneity-of-slopes

FIG. 2.—Larval survival to metamorphosis (mean $\pm$ 1 SE) for five different *Salamandra salamandra* sibships under four different environmental conditions. Treatments were: TF (high temperature-high food), Tf (high temperature-low food), tF (low temperature-high food), tf (low temperature-low food).

(ANCOVA) analysis revealed that the regression lines were not parallel ($F_{2,141} = 20.96$, $P < 0.001$). The slope of treatment tf was lower than the other two treatments, suggesting differences in their growth rates. The slopes of TF and tF did not differ significantly ($F_{1,90} = 0.71$, $P = 0.40$), showing only a difference in their elevations (treatment effect $F_{1,91} = 5.01$; $P = 0.027$; adjusted means TF, 0.603; tF, 0.647). Cross-environmental correlations of metamorphic traits were only significant for size at metamorphosis between environments that

differed in food amounts at low temperature. Mass-specific growth rate and time to metamorphosis were marginally significant after applying the Bonferroni correction (Table 6).

Plasticity in Metamorphic Traits under Variations in Food and Temperature

The MANCOVA indicated that the multiple responses of days at metamorphosis, size at metamorphosis, and mass-specific growth for either food and temperature gradients were

TABLE 1.—Correlation coefficients (Pearson r) between larval size at birth and size at different ages across treatments. Treatments were: TF (high temperature-high food), tF (low temperature-high food), tf (low temperature-low food). Probability levels of correlation coefficients are: bold, $P < 0.00001$; ^a, $P = 0.02$; ^b, $P = 0.0003$; ^{NS}, $P > 0.05$.

Treatments	Weeks									
	2	4	6	8	10	12	14	16	18	20
TF $n = 49$	0.54	0.41	0.36 ^a	0.19 ^{NS}	0.30 ^{NS}	0.23 ^{NS}				
tF $n = 55$	0.86	0.83	0.80	0.72	0.65	0.57	0.49 ^b			
tF $n = 53$	0.89	0.89	0.88	0.81	0.84	0.81	0.79	0.77	0.75	0.61

TABLE 2.—Phenotypic (r_P) and genetic correlations: family mean correlation (r_m) and Restricted Maximum Likelihood estimate (r_G) between larval size at birth and growth and metamorphic traits. Treatments were: TF (high temperature-high food), tF (low temperature-high food), tf (low temperature-low food). Significant correlation values appear in boldface. NS: correlation not significantly different to zero. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Treatments	Metamorphic Traits		
	Size at metamorphosis	Date at metamorphosis	Mass-specific growth
TF	$r_P = 0.06^{NS}$	$r_P = -0.05^{NS}$	$r_P = -\mathbf{0.51}^{**}$
	$r_m = 0.25^{NS}$	$r_m = -0.27^{NS}$	$r_m = -0.30^{NS}$
	$cov_G = 0.004, r_G = 0.14,$	$cov_G = -0.008, r_G = -0.44$	$cov_G = -0.012, r_G = -0.18$
	$\chi^2 = 0.04^{NS}$	$\chi^2 = 0.29^{NS}$	$\chi^2 = 0.039^{NS}$
tF	$r_P = 0.05^{NS}$	$r_P = -\mathbf{0.28}^*$	$r_P = -\mathbf{0.71}^{***}$
	$r_m = 0.40^{NS}$	$r_m = -0.62^{NS}$	$r_m = -\mathbf{0.89}^*$
	$cov_G = 0.012, r_G = 0.40,$	$cov_G = 0.005, r_G = 0.28,$	$cov_G = -\mathbf{0.168}, r_G = -\mathbf{0.93},$
	$\chi^2 = 0.56^{NS}$	$\chi^2 = 0.24^{NS}$	$\chi^2 = 9.55^{**}$
tf	$r_P = \mathbf{0.32}^*$	$r_P = -\mathbf{0.39}^{**}$	$r_P = -\mathbf{0.55}^{***}$
	$r_m = 0.46^{NS}$	$r_m = -0.34^{NS}$	$r_m = -0.64^{NS}$
	$cov_G = \mathbf{0.016}, r_G = \mathbf{0.61}$	$cov_G = -0.001, r_G = -0.11,$	$cov_G = -\mathbf{0.026}, r_G = -\mathbf{0.74},$
	$\chi^2 = 4.59^*$	$\chi^2 = 0.033^{NS}$	$\chi^2 = 5.22^*$

affected by the overall effect of sibships and treatments, but not by the sibship $\times$ treatment interaction, after controlling for the effect of larval birth size (Table 7). Univariate results of two-way mixed model ANCOVA tests of how responses in metamorphosis are affected by sibship and the two environmental gradients (food variation at low temperature and temperature variation at high food levels) were also calculated (Table 7). In the food gradient, variation in food resources affected mass-specific growth, size at metamorphosis, and the length of the larval period. Sibship was also significant for time to and size at metamorphosis for an average food environment, but not for mass-specific growth. However, no significant sibship $\times$ food interaction was found

for any trait with the exception of a marginally significant value for time to metamorphosis (Table 7; Fig. 4). With respect to the temperature gradient, contrasting temperatures affected larval mass-specific growth but explained no significant variation in metamorphic traits. At higher temperatures, salamanders neither increased developmental rate nor was their specific growth rate less than at lower temperatures. This lower growth performance, together with the higher mortality observed within this treatment, suggests that high temperature is either a stressor that affects larval salamander development or that the amount of food provided did not cover all the energetic demands of maintenance and growth (Zakrzewski 1987). A sibship effect was

TABLE 3.—Broad-sense heritability values (H^2) calculated as the proportion of the broad-sense genetic component of variance with respect to the total phenotypic variance. Data are residuals on larval birth size. Components of variance were estimated using the Restricted Maximum Likelihood Quercus program (Shaw and Shaw, 1992) (see Methods for details). Treatments were: TF (high temperature-high food), tF (low temperature-high food), tf (low temperature-low food). Significant nonzero broad-sense heritability values are in bold.

Treatments	Traits			
	Larval size at 10 wk	Date at metamorphosis	Size at metamorphosis	Mass specific growth
TF	$H^2 = 0.19$	$H^2 = 0.24$	$H^2 = 0.42$	$H^2 = 0.01$
	$\chi^2 = 1.14$	$\chi^2 = 1.38$	$\chi^2 = 3.30$	$\chi^2 = 0.005$
	$P = 0.286$	$P = 0.240$	$P = 0.069$	$P = 0.944$
tF	$H^2 = 0.03$	$H^2 = \mathbf{0.94}$	$H^2 = \mathbf{0.87}$	$H^2 = 0.30$
	$\chi^2 = 0.06$	$\chi^2 = 21.35$	$\chi^2 = 17.55$	$\chi^2 = 2.77$
	$P = 0.806$	$P = 0.000004$	$P = 0.00003$	$P = 0.096$
tf	$H^2 = 0.05$	$H^2 = \mathbf{0.49}$	$H^2 = \mathbf{0.80}$	$H^2 = 0.16$
	$\chi^2 = 0.19$	$\chi^2 = 6.10$	$\chi^2 = 13.64$	$\chi^2 = 0.95$
	$P = 0.659$	$P = 0.0134$	$P = 0.0002$	$P = 0.331$

TABLE 4.—Summary of MANOVA repeated measures for growth responses of *Salamandra salamandra* larvae raised in three different environments, with respect to sibship, time, and sibship $\times$ time interaction.

Source of variation	MANOVA		df	P
	Wilk's λ	Rao R		
Treatment TF (high temperature-high food) up to week 10				
Sibship	0.262	2.064	24, 105	0.0064
Time	0.116	527.271	5, 31	<0.00001
Sibship $\times$ time	0.491	1.239	20, 103	0.239
Treatment tF (low temperature-high food) up to week 12				
Sibship	0.292	2.328	28, 160	0.0005
Time	0.017	442.305	6, 45	<0.00001
Sibship $\times$ time	0.628	0.941	24, 158	0.548
Treatment tf (low temperature-low food) up to week 16				
Sibship	0.272	1.662	36, 144	0.019
Time	0.021	228.563	8, 39	<0.00001
Sibship $\times$ time	0.383	1.347	32, 145	0.121

found for size at metamorphosis. No sibship $\times$ temperature interaction was found with the exception of a marginally significant value for mass-specific growth. The assumption that variances remain constant for the testing of sibship $\times$ environment interactions was necessary since an overestimate can occur if among-family variance increases between environments (Fry, 1992). However, this was not the case for the temperature effect on mass-specific growth because variance among sibships was homogeneous (Bartlett's test $\chi^2 = 8.24$; df = 9; $P = 0.51$; Fig. 4).

DISCUSSION

The Effect of Larval Birth Size in Salamandra salamandra

Larval size at birth in fire salamanders affected potentially important components of fitness, such as survival to metamorphosis in high temperature treatments, and correlated positively with larval longevity in the Tf regime (where no individual reached metamorphosis). In addition, variance in the size of larvae over a large part of their aquatic development was

mostly explained by their individual size at birth. The impact of birth size on future larval performance was contingent on larval environments (Semlitsch and Gibbons, 1990). At the slowest growth treatment (low food and temperature), the duration of dependence on birth size was longer than in the other treatments, and it not only affected larval growth but also determined size at metamorphosis.

No information on the sources of variation that control larval size at birth in *S. salamandra* is available. Maternally induced effects can affect larval size at birth directly through egg yolk provision. In addition, the extended embryo retention showed by this species may increase the opportunity for maternal effects through the gestational environment provided by the mother (e.g., health or condition) that may also affect offspring phenotype (Lombardi, 1996). It has been shown that, in some *S. salamandra* populations, larger females produce larger offspring (Sharon et al., 2000; Thiesmeier, 1990; but see Kopp and Baur, 2000; Rebelo and Leclair, 2003), suggesting a maternal-offspring size correlation. In addition, female breeding populations are age

TABLE 5.—Genetic correlations estimated by Restricted Maximum Likelihood (REML), family mean estimates, and phenotypic correlations with associated probabilities between time to and size at metamorphosis of *Salamandra salamandra* larvae raised under three food/temperature regimes. * $P < 0.05$, *** $P < 0.001$.

Treatments	REML	Family mean	Phenotypic
High temperature-high food (TF)	0.964 ($\chi^2 = 59.63^{***}$)	0.897*	0.916*** ($n = 39$)
Low temperature-high food (tF)	0.951 ($\chi^2 = 41.25^{***}$)	0.912*	0.814*** ($n = 55$)
Low temperature-low food (tf)	1.002 ($\chi^2 = 37.24^{***}$)	0.956*	0.763*** ($n = 53$)

FIG. 3.—Covariation of date of metamorphosis and mass at metamorphosis for larvae grown under three temperature/food regimes. Notations as in Fig. 2.

structured (Alcobendas and Castanet, 2000); this characteristic may affect reproductive output such as larval size at birth (Semlitsch and Gibbons, 1990). However, intrinsic genetic factors of larvae may also be involved and may modify larval size at birth. For instance, hatchling size in three anuran oviparous species is largely attributable to maternal effects, but, also, nonadditive genetic variance plays a significant role in its expression (Semlitsch and Schmiedehausen, 1994; M. Tejedo and M. J. Sánchez Herraiz, personal observation; Travis et al., 1987).

Sibship variability in average birth size persists during most of the larval period. The fact that there was no sibship $\times$ time interaction in any particular environment suggests that growth trajectories were parallel across sibships, and, as a result, females that differed in the initial size of their young generated variation in the elevations of the growth curve, which remained unaltered throughout most of larval development. Variation in larval size may be subject to selection since size affects intraspecific aggressive encounters in fire salamanders, i.e., smaller

individuals receive more attacks than larger ones (Reques and Tejedo, 1996). If aggression in larval salamanders functions as a mechanism of spacing or is a form of conspecific predation (cannibalism) as reported in some fire salamander populations (Degani et al., 1980; Thiesmeier, 1992; Warburg, 1992; our personal observations) and other species of salamanders (Kusano et al., 1985; Maret and Collins, 1994), smaller larvae may be at a clear disadvantage.

Maternal effects tended to be greatest during the first weeks of larval growth, declining as the larvae became older (as revealed by the decreasing correlation values between initial larval size and subsequent growth). These became nonsignificant for the TF treatment. However, although maternal effects also declined over larval growth in the lower temperature treatments, they did not vanish entirely and accounted for a significant amount of variation in metamorphic traits. The duration of the larval period was shorter for larvae that were initially larger. This same result was found in a population of *S. salamandra terrestris* from Switzerland (Kopp and Baur,

TABLE 6.—Cross-environmental, broad-sense genetic correlations for time to metamorphosis, mass at metamorphosis and mass-specific growth in *Salamandra salamandra* larvae, based on residuals after removing the effect of individual larval mass at birth. Estimated genetic correlations are the family mean correlation. Associated significance values (whether they are different from zero) appear in brackets. Individual significance values >0.10 are listed as NS. Only values highlighted in boldface are significant after applying a table-wide Bonferroni correction. Treatments were: TF (high temperature-high food), tF (low temperature-high food), tf (low temperature-low food).

	tF	tf
(a) Date to metamorphosis		
TF	0.289 (NS)	0.429 (NS)
tF		0.816 (0.09)
(b) Size at metamorphosis		
TF	0.758 (NS)	0.576 (NS)
tF		0.959 (0.01)
(c) Mass-Specific Growth		
TF	0.523 (NS)	0.385 (NS)
tF		0.892 (0.04)

2000). In addition, size at metamorphosis correlated positively with larval size at birth, both phenotypically and genetically, in the tf treatment. Consequently, the evolution of size at metamorphosis in these environmental conditions was not independent of larval size at birth (assuming that variability in both traits is partially explained by genetic sources). Results obtained in other amphibians suggest a similar pattern of decline over development. Although maternal effects do not seem to determine metamorphic variables in some species (Semlitsch and Gibbons, 1990; Tejedo and Reques, 1992; Travis et al., 1987), hatching size in some species has been found to influence metamorphosis (Parichy and Kaplan, 1992; Semlitsch and Schmiedehausen, 1994).

Maternal effects provided by female *S. salamandra* through larval birth size do not seem to vanish. Rather, they remain important and affect larval survival and other metamorphic traits that confer fitness, such as growth performance during most of the larval period. They even determine the time to and size at metamorphosis when animals are raised at low temperatures. If larval size at birth is basically the result of inherited maternal effects, and not the expression of the animal's own genotype, this maternal inheritance may be adaptive. Maternal effects may have a ge-

netic basis or may be environmentally induced. Insofar as variation in maternal inheritance has a genetic component, a response to selection of maternal effects may occur. If the maternal component is environmentally induced, this response would not occur (Kirkpatrick and Lande, 1989; Lande and Kirkpatrick, 1990). Further investigation is required to ascertain the degree of genetic influence on the size of *S. salamandra* at birth to provide insights into the evolution of reproductive strategies in this species.

Sibship Variation and Plasticity in Metamorphic Traits

Sibship variation in larval size at 10 wk of age, independent of initial birth size, was seen in none of the treatments. This may indicate that size variation is mostly dependent on maternal contributions and not on intrinsic genetic variation. This situation was altered at the moment of metamorphosis, and, after controlling for initial larval birth size, significant broad-sense heritabilities were found for size and the timing of metamorphosis at the two lower temperature treatments. A residual but significant amount of genetic variance was also found for size at metamorphosis in the TF treatment. This shift in the causal factor contributing to the developmental phenotype of salamanders (from maternal factors to their own genotype) seems to be a general pattern in most organisms. Maternal effects are usually more pronounced at the juvenile stages of growth, but generally wane as the offspring come to maturity and their own genotypes take full control (Kearsey and Pooni, 1996). This situation is not, however, universal (see Holtmeier, 2001).

The strong covariation between size and time to metamorphosis was shown by both phenotypic and genetic approaches (see a similar result in Kopp and Baur, 2000 for a *S. salamandra terrestris* population). These traits cannot vary separately and are probably unable to evolve independently. A larger size at metamorphosis could only be attained if larvae develop at a slow rate. This result is also supported by the fact that mass-specific growth rate did not differ across sibships and that the trajectories of larval growth previous to metamorphosis were parallel. The fact that sufficient genetic variance (broad-sense, in-

TABLE 7.—Summary of MANCOVA and univariate two-way mixed model ANCOVA for responses of *Salamandra salamandra* larvae to sibship and food and temperature variation. Response variables were: date to metamorphosis, size at metamorphosis, and size-specific growth. The covariate was larval mass at birth. MS_N mean square of numerator. MS_D mean square of denominator. Treatments were: TF (high temperature-high food), tF (low temperature-high food), tf (low temperature-low food).

Sources of variation Food variation (tF vs. tf)				Sources of variation Temperature variation (TF vs. tF)			
Wilks' Lambda	df 1, df 2	P		Wilks' Lambda	df 1, df 2	P	
Sibships (S)	0.708	12, 333	0.000014	Sibships (S)	0.673	12, 333	0.000001
Environments (E)	0.107	3, 126	<0.000001	Environments (E)	0.840	3, 126	0.000065
S × E	0.904	12, 333	0.380	S × E	0.911	12, 333	0.452
Ln Larval birth size	0.008	3, 126	<0.000001	Ln Larval birth size	0.087	3, 126	<0.000001

Source of variation	Date to metamorphosis				Size at metamorphosis				Size-specific growth			
	MS _N	MS _D	F	P	MS _D	MS _N	F	P	MS _N	MS _D	F	P
Food variation (tF vs. tf)												
Sibship (S)	0.127	0.018	7.259	0.032	0.292	0.013	22.087	0.0016	1.479	1.462	1.011	0.492
Environment (E)	4.365	0.018	246.14	0.0001	1.364	0.013	103.84	0.0001	418.1	1.484	281.68	0.0001
S × E	0.018	0.009	2.009	0.099	0.012	0.017	0.755	0.552	0.609	0.369	1.65	0.168
Ln Larval birth size	0.182	0.009	20.26	0.0001	0.0003	0.017	0.002	0.962	11.251	0.369	33.86	0.0001
Error	0.009				0.017				0.369			
Temperature variation (TF vs. tF)												
Sibship (S)	0.180	0.065	2.755	0.163	0.490	0.064	7.704	0.028	1.329	1.672	0.795	0.582
Environment (E)	0.381	0.066	5.794	0.069	0.147	0.064	2.307	0.196	1.424	1.686	8.441	0.041
S × E	0.067	0.041	1.654	0.169	0.064	0.059	1.091	0.367	1.742	0.737	2.363	0.061
Ln Larval birth size	0.071	0.041	1.749	0.189	0.006	0.006	0.099	0.753	25.091	0.737	34.04	0.0001
Error	0.041				0.006				0.737			

cluding dominance and additive effects) exists for both size at and time to metamorphosis probably indicates that selection does not channel these traits towards a specific phenotypic value. The fire salamander population from the Mijares area breeds in streams that, in some years, dry up at the end of summer, resulting in larval mortality by desiccation. Rapid development would, therefore, appear to be advantageous, but has a cost of smaller size at metamorphosis. The latter has been shown to covary positively with traits that confer fitness via enhanced survival during post-metamorphic life (Newman and Dunham, 1994) and is a predictor of age and size at sexual maturity, which has clear effects on fitness (Berven, 1990; Semlitsch et al., 1988). Slower developers would attain larger sizes at metamorphosis and then reach sexual maturity sooner. The existence of antagonistic pleiotropy in fitness traits, such as that found for size at and time to metamorphosis, is considered a constraint to the evolution of the phenotype due to opposing selection responses (Lande, 1979). However, it seems to be the more fre-

quent pattern in life history traits (Roff, 1997), particularly in the correlation distribution of size at metamorphosis and differentiation rate in amphibians (Berven, 1987; Blouin, 1992a; Newman, 1988; Semlitsch, 1993; Travis et al., 1987; but see Tejedo and Reques, 1992). It is postulated that the antagonism of fitness components often plays a role in maintaining genetic variance and polymorphism (Rose, 1982; but see Curtsinger et al., 1994). In any case, the supposed trade-off may be simply the unavoidable consequence of variation in the duration of larval development, which promotes variation in size at metamorphosis (Blouin, 1992b). The analysis of reaction norms to differing environments revealed the lack of significant sibship × environment interaction for any metamorphic trait under the two environmental gradients. In addition, the family mean genetic correlation for size at metamorphosis with respect to the food gradient indicates a dependence of character states across environments differing in nutrient availability. The rate of evolution of the reaction norm will necessarily depend on the genetic variance

FIG. 4.—Reaction norms for five different *Salamandra salamandra* sibships for larvae grown under three temperature/ food regimes. Notations as in Fig. 2.

within environments in combination with the degree of independence of the character states expressed in different environments. In addition, the absence of a genetic trade-off with other traits that confer fitness is necessary (Via and Lande, 1985). Size at metamorphosis showed significant genetic variability within each environment. However, the fact that character states across food levels are largely correlated on each other, and the existence of a genetic trade-off in fitness with duration of larval period, can reduce the potential for evolution of the reaction norm for size at metamorphosis in differing food level environments.

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SYSTEMATIC REVISION OF THE GENUS *LACHESIS* DAUDIN, 1803 (SERPENTES, VIPERIDAE)

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ABSTRACT: The genus *Lachesis* Daudin, 1803 was partitioned into six geographic groups to evaluate the taxonomic status and phylogenetic relationships among these groups. Characters of external morphology and hemipenial anatomy were evaluated. Results obtained through the phylogenetic and statistical analyses support recognition of the Central American species *L. melanocephala* and *L. stenophrys* as distinct species, whereas the South American *L. muta* is recognized as monotypic, without further differentiation in subspecies. These results provide further evidence of the independence of the Central American and South American lineages and are congruent with other studies based on molecular data.

Key words: Biogeography; *Lachesis*; Phylogeny; Systematics; Taxonomy; Viperidae

THE GENUS *Lachesis* Daudin, 1803 as currently recognized (Zamudio and Greene,

1997) includes: *Lachesis muta* (Linnaeus, 1766), *Lachesis stenophrys* Cope, 1876, and *Lachesis melanocephala* Solórzano and Cerdas, 1986. *Lachesis muta* includes two subspecies: *L. m. muta* (Linnaeus, 1766) and *L. m.*

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rhombeata Wied-Neuwied, 1824. The nominal subspecies is distributed among the equatorial forests of Brazil, Guyana, Venezuela, Trinidad and Tobago, Bolivia, Peru, and Pacific slopes of Ecuador and Colombia (Campbell and Lamar, 1989). *Lachesis m. rhombeata* occurs in the Atlantic Forest, from the Brazilian state of Ceará (Borges-Nojosa and Lima-Verde, 1999) to the state of Rio de Janeiro. *Lachesis stenophrys* is found on the Atlantic versant of Costa Rica, Panama, and southern Nicaragua, while *L. melanocephala* is distributed along the Pacific coast of southeastern Costa Rica (Campbell and Lamar, 1989). The diagnostic external morphological features of the genus include tuberculate, almost pyramidal dorsal scales and the twice-divided distal subcaudal scales (Campbell and Lamar, 1989; Hoge and Romano, 1973). Despite uncertainty of its relationship among New World crotalines, the monophyly of *Lachesis* has been supported by many studies (Gutberlet and Harvey, 2002; Parkinson, 1999; Vidal et al. 1999; Werman, 1992).

Zamudio and Greene (1997) conducted a comprehensive study to estimate phylogenetic relationships within *Lachesis* using mitochondrial DNA. Their results suggest the independence of Central and South American lineages and show that specimens from Mato Grosso, Brazil (*L. m. muta*) are more closely related to the Atlantic Forest specimens (*L. m. rhombeata*) than to individuals from Surinam and Ecuador (*L. m. muta*), suggesting that the current taxonomy needs to be revised.

In this paper, we evaluate the validity of the currently recognized taxa of *Lachesis* using characters related to external morphology and hemipenes, investigate the phylogenetic relationships among some geographic groups, and redefine the geographical distribution of valid taxa.

MATERIALS AND METHODS

Character Description

Morphological data related to squamation, hemipenes, and color pattern were collected from 265 alcohol-preserved specimens (Appendix I). Museum acronyms follow Leviton et al. (1985), with the addition of Universidade Federal do Ceará, Fortaleza (UFC); Instituto

Vital Brazil, Niterói (IVB); and Instituto Clodomiro Picado, San Jose, Costa Rica (ICP). Terminology follows Gutberlet (1998) for morphological features, Klauber (1972) for squamation, and Dowling and Savage (1960) and Myers and Campbell (1981) for hemipenes. The characters were selected from those used by Campbell and Solórzano (1992), Gutberlet (1998), Werman (1992), and Zamudio and Greene (1997). Character descriptions are given as follows.

1. Number of anterior dorsal scale rows (AD), counted at a position one head length posterior of the quadrate-mandibular articulation
2. Number of middorsal scale rows (MD), counted at midbody
3. Number of posterior dorsal scale rows (PD), counted at a position one head length anterior of the medial portion of cloaca
4. Number of ventrals (VE), counted following the method of Dowling (1951)
5. Number of subcaudals (SC), counted following the method of Peters (1964) and including those that are twice-divided
6. Number of supralabials (SL), counted following Gutberlet (1998)
7. Number of infralabials (IL), positioned between the mental scale and the posterior corner of the mouth (rictal region)
8. Prelacunal and second supralabial scale (PS), not fused (a) or fused (y)
9. Number of subfoveal rows (SF), defined according to Klauber (1972)
10. Number of intersupraoculars (IS), counted in a straight line from one supraocular to the other (supraoculars not included)
11. Number of suboculars (SO), scales below the eyes that contact the orbit
12. Number of interoculolabials (IO), counted according to Klauber (1972), including one supralabial and one subocular
13. Number of prefoveals (PF), bordered by the supralabials, nasals, loreals, lacunals, and subfoveal scales (if present)
14. Number of canthals (CA), counted according to Gutberlet (1998)
15. Number of interrials (IR), counted according to Gutberlet (1998)

16. Loreal scale (LO), entire (0) or fragmented (1); positioned between the superior preocular scale and the postnasal scale
17. Rostral scale (RO), higher than broad (0) or broader than high (1); positioned between the first pair of supralabials
18. Number of preoculars (PO), one (0) or two (1); positioned in front of the ocular orbit
19. Preocular/supralacunal scales (PO/SUL), fused (0) or separated (1)
20. Parietals (PA), keeled (0) or tuberculate (1)
21. Internasals (IN), larger than the adjacent scales (0) or not distinct (1)
22. Prenasals (PNA), small (0) or large (1)
23. Postnasal (PON), contacts first supralabial (0) or no contact (1)
24. Sublacunal (SLC), entire (0) or divided (1); positioned posterior to the prelacunal scale (anteriormost scale inside the loreal pit)
25. Chinshields (CS), counted including the first pair of infralabials
26. Chinshields-infralabials (CS-IL), the lowest number of gular scales rows between the first scale after chinshields and the infralabial scales, examining both sides
27. Number of gulars between the chinshields and the first ventral (GV), the fewest scales between the first gular after chinshields and the first ventral scale (for this character the first ventral was considered to be the first one that was wider than long)
28. Width of the postocular stripe (WP), number of scale rows contained by the stripe at it broadest portion
29. Basal and lateral spines (HS), less than 15 spines per lobe (0); 25 to 50 spines per lobe (1); more than 70 spines per lobe (2)
30. Calyces on lateral surfaces of hemipenial lobes, restricted to distal portion (0); extending proximally to level of crotch (1)
31. Dorsal color of head, head with well defined dark blotches (0); head with inconspicuous dark blotches (1); head with practically no pigment, only some sparse dark punctations (2); head completely dark (3)
32. Color pattern of dorsal blotches along the body, dorsal blotches forming vertical bars laterally (0); not forming vertical bars laterally (1)

Statistical Analysis

For purposes of this analysis, the genus *Lachesis* was partitioned into six geographic groups, corresponding to specimens located on the Atlantic ($n = 29$) and Pacific ($n = 7$) versants of Central America, north ($n = 37$) and south of the Amazon River ($n = 68$), in Mato Grosso (Brazil) ($n = 56$), and within the Atlantic Forest of Brazil ($n = 68$). The Central American groups were defined based on current taxonomy and on a particular natural barrier (cordillera of Talamanca) that exerts influence on their distribution (Zamudio and Greene, 1997). Specimens from the Atlantic Forest and Amazonian region correspond to the currently recognized subspecies of South American *Lachesis* (Campbell and Lamar, 1989; Zamudio and Greene, 1997). Based on the results of Zamudio and Greene (1997), we consider specimens from Mato Grosso as a distinct group. We defined groups from north and south of the Amazon River to evaluate whether this natural barrier has played any role in the diversification of *Lachesis*.

In this study, analysis of specimens from northwestern South America, on the Pacific coast of Ecuador and Colombia (the Chocó region), was not possible. A single specimen from the province of Darien, Panama, was examined and shows features similar to *L. muta*. The results of Zamudio and Greene (1997) concerning specimens from the Chocó region are inconclusive. Nevertheless, Campbell and Lamar (2004) recognize the groups from northwestern South America as a distinct species, *L. acrochorda* (García, 1896), and suggest that this fourth species may be closely related to *L. muta*.

Principal components analysis (PCA; Manly, 2000) was performed to evaluate the distribution of the specimens in multivariate space without a priori definition of the groups, whereas multivariate analysis of variance (MANOVA; Zar, 1999) and discriminant analysis (Manly, 2000) were employed to study the variation between and within each previously defined group. Orthogonal comparisons (Sokal and Rohlf, 1995) among some groups were performed. The MANOVA was further used to verify the existence of sexual dimorphism within each group. Characters

that showed insufficient variation to justify the assumption of normality were not included in the statistical analysis. The following 13 characters were employed: number of anterior, middorsal, and posterior dorsal scale rows (three counts); ventral scales; subcaudals (including those that are twice-divided); supralabials; infralabials; intersupraoculars; interrials; chinshields; chinshields-infralabials; gulars between the chinshields and the first ventral; and width of postocular stripe. In a later analysis, the South American specimens were classified, independent of their origin, based only on dorsal color of the head (character 31), and the new groups were subjected to discriminant analysis. This criterion was used because South American subspecies are diagnosed according to this feature (Campbell and Lamar, 1989; Hoge, 1966). Specimens from Mato Grosso were identified on the discriminant graph to compare their distribution in discriminant space to the currently recognized subspecies. In all analyses, assumptions of univariate normality and homoscedasticity were evaluated with the Kolmogorov-Smirnov's test and Levene's test, respectively (Zar, 1999). To test the robustness of the results obtained, we used the bootstrap method (Manly, 1991) with 1000 pseudoreplications using MatLab 4.2c1 for Windows (Mathworks, 1994). All statistical tests were performed with a significance level of 0.05, except the orthogonal comparisons (significance level of 0.01) in which a conservative position was adopted (following Sokal and Rohlf, 1995). The 95% confidence limits of the statistically significant variables in the multivariate variance analysis were compared among the different geographic groups to evaluate the level of overlap. Variables were considered taxonomically informative when the confidence limits did not overlap.

Phylogenetic Analysis

A phylogenetic matrix containing 26 morphological characters selected from the list above was analyzed. The tree was rooted with the outgroup method (Nixon and Carpenter, 1993). Outgroup taxa were *Bothriechis lateralis*, *B. nigroviridis*, and *Atropoides nummifer*. These taxa were chosen based on the analyses

of Werman (1992), Zamudio and Greene (1997), and especially Parkinson (1999). Characters that showed no variation in both ingroup and outgroup (17–19; 23–24) were not used. Overlapping meristic characters (1–7; 9–15; 25–28) and qualitative polymorphic characters (8) were coded by the generalized frequency coding (GFC) method (Smith and Gutberlet, 2001), using the program Code This! (Gutberlet et al., 2000). The method incorporates features of the frequency bins (Wiens, 1995) and gap weighting (Thiele, 1993) methods. The GFC method divides polymorphic characters into subcharacters, which allows coding of the entire frequency distribution and, consequently, incorporates more information in the analysis compared to previous methods (Smith and Gutberlet, 2001). We used the USW (unequal subcharacter weighting) option of Code This! to weight the subcharacters. Subcharacter weights were adjusted according to Smith and Gutberlet (2001) so that equal weights were assigned to conventionally coded and generalized frequency coded characters. Binary and multistate (non-meristic) characters were treated as unordered. Characters coded by the GFC method were treated as ordered. A GFC matrix included a total of 284 characters (7 conventional and 277 subcharacters), of which 95 were uninformative and, thus, excluded from analysis. The GFC matrix is not presented due to space considerations, but can be obtained from the authors. In a second analysis, the polymorphic characters were coded by the gap weighting method (Thiele, 1993) using the median of each character (Table 1) for each taxon to avoid the effect of outliers in the analysis. The resulting matrix (Table 2) included the original 26 morphological characters, although Character 25 (chinshields) was not informative. A third analysis combined our morphological data with mtDNA sequence data from Zamudio and Greene (1997). The combined analysis matrix contained 812 characters, of which 254 were parsimony-informative.

We performed parsimony analyses (Farris and Kluge, 1986; Kluge and Farris, 1969) using PAUP* 4.0b8a for Macintosh (Swofford, 2001) to infer a hypothesis of phylogenetic relationships for the genus *Lachesis*. The six geographic groups defined in the statistical analyses were treated as terminal taxa. The

The MANOVA between all groups was significant for females ($F = 12.4026$; $P < 0.01$; $n = 110$) and males ($F = 14.2202$; $P < 0.01$; $n = 155$). An orthogonal comparison between south and north Amazon River specimens was not significant for females ($F = 2.4582$; $P = 0.05$; $n = 38$), but the difference was significant for males ($F = 4.2598$; $P < 0.01$; $n = 67$). The group from Mato Grosso was significantly different from the sample formed by specimens from north and south of the Amazon River for females ($F = 8.2261$; $P < 0.01$; $n = 58$) and males ($F = 12.0246$; $P < 0.01$; $n = 103$). The Atlantic Forest group was significantly different from the sample formed by specimens from north and south of the Amazon River for females ($F = 18.7199$; $P < 0.01$; $n = 73$) and males ($F = 20.8826$; $P < 0.01$; $n = 100$). The specimens from Mato Grosso and from the Atlantic Forest were significantly different for females ($F = 8.4516$; $P < 0.01$; $n = 55$) and males ($F = 14.4941$; $P < 0.01$; $n = 69$).

The results of the PCA were similar to the discriminant analysis and, therefore, are not shown. Discriminant analysis differentiated the groups of females and males through the first two discriminant functions, which corresponded to 89% of the entire variation. The combination of the third discriminant function, with the first or second functions, showed no satisfactory discrimination in either sex. To visualize the level of differentiation among groups, the obtained scores were plotted, and both graphs show a clear differentiation between South and Central American groups (Fig. 1). Groups from Amazonia and Atlantic Forest are differentiated from each other, but specimens to the north and south of the Amazon River are poorly distinguished, supporting the results of the MANOVA. Specimens from Mato Grosso occupy a position between Amazonian and Atlantic Forest groups.

In the third analysis, based on the dorsal color of the head, the canonical plot graphs show discrimination between the two existing patterns in both sexes (Fig. 2). The first two discriminant functions represented 95% of the variation in females and 98% in males. Nevertheless, specimens from Mato Grosso are scattered along both axes, not forming an intermediate group as in the previous dis-

criminant analyses (Fig. 1). Furthermore, there is no congruence between the localities of the specimens from Mato Grosso and the position they occupy in the graph, i.e., geographically close localities are not necessarily proximal in the graph. Because specimens from Mato Grosso do not show any correlation between head-color pattern and geographical distribution in the discriminant space (Fig. 2), it is not possible to diagnose these two putative subspecies based on head coloration.

Phylogenetic Analysis

The GFC analysis yielded a single most-parsimonious tree with 1,144,536 weighted steps, consistency index of 0.744, and rescaled retention index of 0.563 (Fig. 3). The gap weighting analysis generated a single most-parsimonious tree with 820 weighted steps, consistency index of 0.789, and rescaled retention index of 0.624, showing a topology identical to the tree recovered by GFC (Fig. 3). The combined data analysis recovered five most-parsimonious trees with 3,034,042 weighted steps, consistency index of 0.817, and rescaled retention index of 0.612. The strict consensus of these trees is similar to the trees recovered by the other analyses, the ambiguities being restricted to relationships among the outgroups (Fig. 3). The following synapomorphies are relative to morphological data supported under both GFC and gap weighting coding methods, under ACCTRAN and DELTRAN optimization criteria. The clade formed by the genus *Lachesis* is supported by three unambiguous synapomorphies relative to the outgroup selected: number of intersupraoculars, tuberculate parietals, and more than 70 spines per lobe on the hemipenis. The clade formed by the South American groups of *Lachesis* is supported by two unambiguous synapomorphies: internasals distinct from adjacent scales, and dorsal blotches not forming vertical bars laterally. The clade formed by the Central American groups is supported by one unambiguous synapomorphy under the ACCTRAN criterion of optimization: dorsal blotches forming vertical bars laterally. Atlantic Forest, Mato Grosso, and south Amazon River groups form a clade supported by one unambiguous synapomorphy: number of intersupraoculars.

FIG. 1.—Canonical analysis of the six female and male geographic groups of *Lachesis* with the respective 95% confidence limits ellipse for each group.

The clade formed by the Mato Grosso and Atlantic Forest groups is supported by one unambiguous synapomorphy: head generally with well defined dark blotches. None of the South American geographic groups have autapomorphies. The group from the Pacific coast of Central America, *L. melanocephala*, is supported by one autapomorphy: head completely dark. The group from the Atlantic coast of Central America, *L. stenophrys*, is supported by one autapomorphy under the DELTRAN optimization criterion: head with practically no pigment, showing only some sparse dark punctation.

Geographic Variation in *Lachesis*

The general morphological features that distinguish the groups of *Lachesis* are readily apparent. The Atlantic Forest group has slightly fewer ventral scales than the Amazonian and Mato Grosso groups. Specimens from the Atlantic Forest have a wider postocular stripe. The prelacunal and second supralabial scales are not fused in the Atlantic Forest

FIG. 2.—Canonical analysis of the female and male geographic groups of *Lachesis* based on the dorsal color pattern of the head. Atlantic Forest and Amazonian patterns correspond to states 0 and 1 of Character 31, respectively. Atlantic Forest MT and Amazonian MT correspond to specimens from Mato Grosso, Brazil, with states 0 and 1 of Character 31, respectively.

group, and this character is variable in other groups. The north Amazon group possesses a slightly narrower postocular stripe compared to other groups. Specimens from Mato Grosso have a wider postocular stripe than Amazonian groups. Central American groups have fewer ventral scales than South American groups. However, with the exception of ventral scales, these features are not completely fixed in these groups and show a high degree of overlap (Table 3). Another relevant feature, though variable, is that members of the Atlantic Forest and Mato Grosso groups have more conspicuous blotches on the dorsum of the head than do Amazonian groups. Central American specimens can be easily diagnosed by the color pattern of the head. The Atlantic Coast group possesses a head that almost completely lacks black pigmentation, while specimens from the Pacific Coast have a completely black head.

DISCUSSION

The statistical analysis distinguished both Central and South American groups of *Lachesis*. South American subspecies are distinct according to some characters (Table 3), but specimens from Mato Grosso are intermediate. The Mato Grosso group is phylogenetically more closely related to the Atlantic Forest group (Fig. 3). However, in the discriminant analysis, data points from Mato Grosso are distributed among Amazonian and Atlantic Forest groups, precluding an unambiguous diagnosis. The characters Color of the Dorsum of the Head and Width of Postocular Stripe, commonly used to distinguish the South American subspecies according to Wied-Neuwied (1824) or Hoge (1966) and Hoge and Romano (1978), are variable in all geographic groups.

Most of the characters we used in the phylogenetic analysis are of the type usually avoided due to the variation that they exhibit in the terminal taxa (Thiele, 1993). These characters (quantitative and polymorphic) are normally excluded from phylogenetic analysis for a variety of reasons, e.g., they show intraspecific variation (Gensel, 1992), are arbitrarily defined (Cox and Urbatsch, 1990), or are highly homoplastic (Campbell and Frost, 1993; Wiens, 1995). Nevertheless, these types of characters are common and are occasionally used in studies, such as ours, that aim to examine inter-intraspecific variation (Campbell and Frost, 1993; Gutberlet, 1998; Wiens, 1999, 2000). Moreover, the ideal character concept varies according to the author, and the criteria for choosing and excluding characters are generally unclear (Poe and Wiens, 2000). Although some criticism of this type of character may be valid (Crother, 1990; Farris, 1966; Mayr, 1969), recent studies corroborate that these characters do have phylogenetic information and should be considered (Gutberlet, 1998; Gutberlet and Harvey, 2002; Smith and Gutberlet, 2001; Wiens, 1995, 1998). The criticism of arbitrary coding of quantitative character states is a problem that may also influence qualitative characters; furthermore, most morphological characters describe quantitative variation, regardless of whether they are coded

FIG. 3.—Most-parsimonious tree recovered for the geographic groups of *Lachesis*. Numbers along the branches represent bootstrap values from the three analyses performed. Bootstrap values were estimated from 1000 pseudoreplicates and are listed (from top to bottom) for combined data, GFC method, and gap weighting method. MT = Mato Grosso; SA = south Amazon River; AF = Atlantic Forest; NA = north Amazon River.

qualitatively or quantitatively (Poe and Wiens, 2000; Wiens, 2001). The GFC method simply translates the frequency distributions of the characters into a set of discrete states, avoiding the use of central tendency measures that could be labeled arbitrary (Smith and Gutberlet, 2001). The GFC method seems to be the most appropriate for treating this problem. Another relevant consideration is whether or not to order the multistate characters (Hauser and Presch, 1991; Kluge, 1991). Gap weighting (Thiele, 1993) and frequency bins (Wiens, 1995) demand that characters be ordered, which may not be a problem if, as Slowinski (1993) argues, the ordering of characters rarely causes changes in the tree topology. The GFC method allows the use of ordered or unordered characters (Smith and Gutberlet, 2001).

The three phylogenetic analyses yielded trees with similar topology (Fig. 3). In general, the bootstrap proportions in the analysis that used GFC are greater than in gap weighting (Fig. 3). Also, the number of clades supported with bootstrap proportions of 70% or greater means, according to Hillis and Bull (1993), great probability of accuracy. These results are similar to a previous study comparing some parsimony coding methods (Smith and Guterlet, 2001) and provide additional evidence

TABLE 3.—Variation in the taxonomically informative variables used to differentiate the geographic groups of *Lachesis*. Data from males and females are grouped. Amazonian groups are analyzed together. VE = number of ventral scales; WP = width of the postocular stripe.

Variables	Mato Grosso (<i>n</i> = 56)		Amazonian (<i>n</i> = 105)		Atlantic Forest (<i>n</i> = 68)	
	95% confidence limits	Amplitude of variation	95% confidence limits	Amplitude of variation	95% confidence limits	Amplitude of variation
VE	224–227	220–232	226–228	219–236	218–222	213–231
WP	2.5–3	1.5–3.5	1.5–2	1–2.5	2.5–3	1.5–4

that GFC can be used to accurately reconstruct phylogeny. Concerning the combined data, bootstrap proportions are generally greater than in the first two analyses, indicating compatibility between morphological and molecular data. The tree topology obtained in our study is congruent with the results of Zamudio and Greene (1997), based on molecular data, and supports the use of quantitative and polymorphic characters in phylogenetic analysis. In our study, these characters were phylogenetically informative and corroborated previous hypotheses derived from independent sources of data.

The results of the phylogenetic analysis combined with statistical data suggest independence between the South and Central America clades, as suggested by Zamudio and Greene (1997). Although the Central American clade is supported by just one unambiguous synapomorphy under AC-CTRAN optimization, the South American clade is supported by two unambiguous synapomorphies under both optimization criteria. The taxonomic status of the two currently recognized Central American species is supported by our study.

The results for South American *Lachesis* suggest that the groups from Mato Grosso and the Atlantic Forest are more closely related to each other than to the Amazonian groups. Zamudio and Greene (1997) also obtained this result, corroborating our conclusion. The north Amazon group seems to have been the first to diverge, being the sister group of the remaining South American groups. These conclusions have relevant implications for currently recognized South American taxa. *Lachesis m. muta* is distributed throughout the Amazon basin, including Mato Grosso. *Lachesis m. rhombeata* is restricted to the Atlantic Forest. According to the cladogram

(Fig. 3), *L. m. muta* is paraphyletic, because specimens from Mato Grosso are more closely related to *L. m. rhombeata* than to *L. m. muta*. Considering the specimens from Mato Grosso as *L. m. rhombeata* would not solve this problem, as *L. m. muta* remains paraphyletic, because the Amazonian groups are not monophyletic according to this analysis. The alternative for making the two taxa monophyletic is to restrict the distribution of *L. m. muta* to latitudes north of the Amazon River and extend the distribution of *L. m. rhombeata* from the Atlantic Forest to south of the Amazon basin. Nevertheless, the north Amazon group has no features that unambiguously distinguish it from the others. Statistical analysis reveals that this group is very similar to the south Amazon group and to specimens from Mato Grosso. In the phylogenetic analysis, this group has no unambiguous autapomorphies. Although the postocular stripe is generally thinner compared to other groups, the degree of overlap is large and a diagnosis of this taxon becomes difficult. The same reasoning prevents the recognition of other South American groups as distinct species.

A hypothesis that may explain the results obtained in our study for South American groups of *Lachesis* is that, during the Pleistocene, the Neotropical region had alternations of moist/hot and dry/cool eras (Haffer, 1969; Van der Hammen, 1972, 1974). These events caused successive expansions and retractions of the forest (Ab'Saber, 1977a; Van der Hammen and Asby, 1994). Bigarella and Andrade-Lima (1982) proposed, based on the morphoclimatic domains of Ab'Saber (1977b), that the Amazonian Forest and the Atlantic Forest were in contact during some period in the Quaternary through the region currently occupied by the "Caatinga." De Vivo (1997)

postulated that this connection between Amazonian and Atlantic forests was capable of sheltering arboreal species of mammals that are typical of tropical forests. The same connection probably allowed some species, which at present have disjunct distributions in the Amazonian and Atlantic forests, to have contiguous populations during the Quaternary, thus curtailing allopatric speciation (De Vivo, 1997). Costa (2003) argued that Central Brazilian forests are fundamental to explanations of the distribution patterns of some lowland small mammals. Nevertheless, there are no fossil records of *Lachesis* from the "Caatinga" domain. All that can be suggested is that the Amazonian and Atlantic Forest groups of *Lachesis* somehow maintained gene flow that prevented a total differentiation between them and that this connection probably occurred through the region now known as Mato Grosso.

Based on these results, we can state that the examined South American groups have morphological differences among them; however, these differences are not sufficiently fixed (Davis and Nixon, 1992) to distinguish two or more independent lineages in the genus *Lachesis* in South America. The taxonomic status of the two currently recognized subspecies is herein altered in order to work solely with monophyletic taxa. Thus, the subspecies *Lachesis muta rhombeata* Wied-Neuwied, 1824 is placed in the synonymy of *Lachesis muta* (Linnaeus, 1766).

TAXONOMY

Taxonomic History

Linnaeus (1766) described *Crotalus mutus* with the type locality as Surinam. Daudin (1803) described the genus *Lachesis* to accommodate the species *C. mutus* Linnaeus, 1766. Wied-Neuwied (1824) described *Lachesis rhombeata* and designated Brazil as the type locality. Cope (1876) described *Lachesis stenophrys* from the type locality of Sipúrio, Costa Rica. Boulenger (1896) placed *L. rhombeata* and *L. stenophrys* in the synonymy of *L. muta*. Taylor (1951) recognized the validity of *Lachesis muta stenophrys* Cope, 1876 and, by fiat, of *L. m. muta* (Linnaeus, 1766) as the nominal subspecies. Hoge (1966) described *Lachesis m. noctivaga*, with the type

locality as the city of Vitória, state of Espírito Santo, Brazil. Hoge and Romano (1978) placed *L. m. noctivaga* in the synonymy of *L. muta rhombeata* and restricted the type locality of the latter taxon to Vitória, Espírito Santo. Solórzano and Cerdas (1986) described *Lachesis m. melanocephala* with the type locality of southeastern Costa Rica. Zamudio and Greene (1997) elevated *L. m. stenophrys* and *L. m. melanocephala* to species level, while maintaining the taxonomic status of South American taxa.

Taxonomic Account

Lachesis Daudin, 1803

- 1803 *Lachesis* Daudin, Bull. Sci. Soc. Philomath. Paris (2)3:188. Type-species: *Crotalus mutus* Linnaeus, 1766
 1811 *Trigonocephalus* Oppel, 1811 [dated 1810], Ann. Mus. Hist. Nat., Paris 16:388. Latreille, 1801. Type-species: *Scytale amodytes* Latreille, 1801
 1815 *Trigalus* Rafinesque, Analyse Nat. (Herpetol. section):77. [Replacement name for *Trigonocephalus* Oppel]
 1820 *Cophias* Merrem, Tent. Syst. Amph., 191:154. Type-species: *Cophias crotalinus* Gmelin, 1788

Diagnosis.—The only oviparous New World crotaline genus. Dorsal scales verrucate, parietals tuberculate, hemipenes with more than 70 spines per lobe, and twice-divided distal subcaudals.

Description.—Large snakes reaching approximately 3 m total length (TL). Head distinct from body. Body in cross section triangular or round. Head scales granular. Rostral triangular, broader than high; nasals divided; prenasals large, postnasals usually not touching first supralabial, internasals distinct or not; usually three small canthal scales; single, elongate postocular; 10–18 intersupracoculars usually not keeled; tuberculate parietals; 26–35 interrials; 8–11 supralabials, third larger; 12–16 infralabials, first pair in contact behind the mental; first two pairs of infralabials usually touch first pair of chinshields; 0–6 prefoveals; 0–1 subfoveal row; sublacunal entire; lacunolabial present or absent; loreal undivided, approximately as high as broad and contacting anterior margin of upper preocular;

two preoculars, longer than high, and bordering the loreal pit; inferior preocular fused to supralacunal; upper preocular contributing to canthus rostralis and larger than inferior preocular; 5–6 interoculolabials; eye relatively small, distance between tangent to ocular globe and tangent to rostral 3–3.5 times eye diameter; vertical pupil; dorsal scale formula 30–42/29–41/22–28 rows; dorsals longer than wide at midbody, becoming almost as long as wide on distal portion of body; dorsals without apical pits and scales from vertebral region strongly keeled, verrucate; this feature less conspicuous in paraventral region where dorsal scales may lack keels; 197–236 ventrals; cloacal scale undivided; 41–56 subcaudals; initial pairs may be entire, most are divided whereas distally the subcaudals are twice-divided.

Color in preservative.—Dorsum of head creamish yellow or brown with black or dark brown blotches with different levels of fragmentation. Black postocular stripe varying from 1–4 rows wide. Body creamish yellow with a brighter distal portion. Dorsal, dark brown to black rhomboidal blotches along the body (18–35), sometimes blotches acquiring a triangular or zig zag aspect. Laterally these blotches may grow narrower, forming vertical bars or maintaining the rhomboidal aspect. Eventually these blotches extend to the ventral scales. Ventrally the head and body are ivory white without blotches.

Geographic distribution.—South American Amazonian forests and Atlantic Forest in Brazil. Central America, in southern Nicaragua, Costa Rica, and Panama (Campbell and Lamar, 2004; Fig. 4). Usually inhabits primary forests; sometimes found in secondary forests.

Lachesis muta (Linnaeus, 1766)

- 1766 [*Crotalus*] *mutus* Linnaeus, Systema Naturae, Ed. 12:373. Type-locality: Surinam
- 1789 *Coluber crotalinus* Gmelin, Systema Naturae, Ed. 13(1):1094. Type-locality: not given
- 1801 *Scytale catenatus* Latreille, In Sonnini and Latreille, Hist. Nat. Rept. 3:162. Type-locality: Surinam
- 1802 *Scytale ammodytes* Latreille, In Sonnini and Latreille, Hist. Nat. Rept. 3:165. Type-locality: Ceylon [In error]
- 1802 *Coluber alecto* Shaw, Gen. Zoology, Amphibians, 3(2):405. Type-locality: Ceylon [In error]
- 1803 *Lachesis mutus*; Daudin, Hist. Nat. Rept. 5:351
- 1803 *Lachesis ater* Daudin, Hist. Nat. Rept. 5:354. Type-locality: Surinam
- 1811 *Trigonocephalus ammodytes*; Oppel, Ann. Mus. Hist. Nat., Paris 16:390
- 1820 [*Cophias*] *crotalinus*; Merrem, Tent. Syst. Amph., 191:144
- 1822 *Trigonoceph[alus] crotalinus*; Schinz, Das Thierreich Grund. Naturgesch. Ver. Anat. 2:144
- 1822 *Lachesis muta*; Schinz, Das Thierreich Grund. Naturgesch. Ver. Anat. 2:144
- 1822 *Lachesis atra*; Schinz, Das Thierreich Grund. Naturgesch. Ver. Anat. 2:144
- 1822 *Scytale catenata*; Schinz, Das Thierreich Grund. Naturgesch. Ver. Anat. 2:144
- 1824 *Bothrops surucucu* Wagler, in Spix, Spec. Nov. Serp. Brazil:59, pl. 23. Type-locality: Brazil
- 1824 *Lachesis rhombeata* Wied-Neuwied, Abbild. Nat. Brazil, Lief. 5:pls. 5, 5a. Type-locality: Brazil
- 1825 C[*rasedocephalus*] *crotalinus*; Gray, Ann. Philos. (2)10:205
- 1854 *Lachesis mutus*; A.-M.-C. Duméril, Bibron, and A.-H.-A. Duméril, Erp. Gén. 7(2):1486
- 1872 *Trigonocephalus (Lachoesis) brasiliensis* Liais, Clim. Géol., Faune Géogr. Brésil: 306–307. Type-locality: not given, probably Rio Doce, MG, Brazil (Hoge and Romano, 1978)
- 1872 *Trigonocephalus rhumbeatus*; Liais, Clim. Géol., Faune Géogr. Brésil:306
- 1872 *Lachoesis rhumbeata*; Liais, Clim. Géol. Faune Géogr. Brésil:306
- 1896 *Lachesis mutus*; Boulenger, Cat. Sn. Brit. Mus. 3:534
- 1898 *Lachesis muta*; Boettger, Kat. Rept. Samml. Mus. Senckenb. Naturforsch. Ges. 2:137
- 1951 *Lachesis muta muta*; Taylor, Univ. Kansas Sci. Bull. 34:184. Type-locality: Surinam
- 1966 *Lachesis muta noctivaga* Hoge, Mem. Inst. Butantan, 32:162, pl. 20 (1965). Type-locality: Vitória, ES, Brazil
- 1978 *Lachesis muta rhombeata*; Hoge and Romano, Mem. Inst. Butantan, 40/41:54

FIG. 4.—Localities of specimens examined of the genus *Lachesis*. Key: *Lachesis muta* ●; *Lachesis melanocephala* ■; *Lachesis stenophrys* ▲; *Lachesis* from Darien, Panamá ▼.

(1976/77). Type-locality: Restrict to Vitória, ES, Brazil, in this work

Diagnosis.—Distinguished from *L. stenophrys* and *L. melanocephala* by having more ventral scales (215–234 in females and 213–236 in males), compared to 197–211 in females and 198–204 in males of *L. stenophrys* and 211–214 in females and 209–214 in males of *L. melanocephala*. Additionally, the head of *L. muta* is not uniformly black as in *L. melanocephala*, but has dark blotches over much of its surface; the head of *L. stenophrys* is nearly immaculate. The dorsal blotches in *Lachesis muta* do not form vertical bars laterally as in *L. stenophrys* and *L. melanocephala*.

Description.—Largest specimen a male 2510 mm SVL and 216 mm TL; body round in cross section; postnasals usually not touching first supralabial; internasals distinct; 3–5 canthals; 11–15 intersupraoculars in females and 10–18 in males; 26–35 interrials in both

sexes; 9–11 supralabials in females and 8–11 in males; 13–16 infralabials in both sexes; 1–6 prefoveals in females and 1–5 in males; 0–1 row of subfoveals; lacunolabial present or absent; 8–14 chinshields in females and 6–14 in males; chinshields separated from infralabials by 5–10 scales in females and 5–11 in males; 3–13 gulars between chinshields and first ventral in females, 4–13 in males; dorsal scale formula 33–41/31–41/23–28 and 30–40/29–38/22–27 in females and males, respectively; 215–234 ventrals in females and 213–236 in males; 41–53 subcaudals in females and 44–56 in males; 9–20 rows of twice-divided subcaudals in females and 9–22 in males.

Color in preservative.—Dorsum of head creamish yellow or brown, with dark brown or black blotches with different levels of fragmentation, varying from dark dots along the head forming small blotches or lines mainly on the posterior portion of the head, enhancing the granular appearance of the dorsum of

head, or head with conspicuous blotches forming markings that contrast greatly with ground color. Black postocular stripe varying from 1–4 rows in width in females and 1–3.5 in males. Dark brown or black rhomboidal dorsal blotches varying from 24–35 in females ($n = 45$) and 18–33 in males ($n = 74$), not forming vertical bars laterally and usually extending to the second or third dorsal scale row; blotches sometimes reaching ventral scales.

Hemipenis.—Usually extending 8–10 subcaudals; bilobed, lobes larger than the hemipenial body that laterally has two basal depressions; sulcus spermaticus divides approximately at midlength, between the bottom of hemipenial body and the point of bifurcation of the lobes and is centrolineal; hemipenis naked proximally; region above the sulcus spermaticus bifurcation is densely spinose (approximately 95–108 spines; $n = 12$); internal surface of lobes has a larger number of spines compared to external surface, which also has larger spines; apical region calyculate, calyces of medial surface of each lobe extend more distally compared to calyces of lateral surface; on hemipenial asulcate side, adjacent to the bifurcation of lobes, tiny spines present.

Geographic distribution.—Occurs in tropical forests of Brazil, Guyanas, Venezuela, Trinidad, Bolivia, Peru, Ecuador, and Colombia. Pacific slopes of Ecuador, Colombia, and province of Darien, Panama. Atlantic Forest in Brazil from the state of Ceará to the state of Rio de Janeiro. Moreover, occurs in the states of Amazonas, Pará, Amapá, Roraima, Rondônia, Acre, Mato Grosso, and Goiás (Campbell and Lamar, 2004; Fig. 4).

Lachesis stenophrys Cope, 1876

- 1876 *Lachesis stenophrys* Cope, Journ. Acad. Nat. Sci. Phila. 8(2) (1875):152. Type-locality: Sipúrio, Costa Rica
 1896 *Bothrops achrocordus* Garcia, Ofidios Venen. Cauca:22, pl. 4. (but see discussion)
 1951 *Lachesis muta stenophrys*; Taylor, Univ. Kansas Sci. Bull. 34:184
 1997 *Lachesis stenophrys*; Zamudio and Greene, Biol. J. Linn. Soc. 62:436

Diagnosis.—Distinguished from *L. melanocephala* by having head that is predominantly unpigmented dorsally. Furthermore, *L. stenophrys* has fewer ventral scales (197–211 in females; 215–234 in males) than *L. muta*.

Description.—Largest specimen a male 2050 mm SVL and 198 mm TL; body somewhat triangular in cross section; postnasals not touching first supralabial; internasals distinct or not; 3–5 canthals; 10–12 intersupraoculars in females and 10–13 in males; 26–31 interriectals in females and 27–31 in males; 8–9 supralabials in females and 8–10 in males; 12–15 infralabials in females and 13–15 in males; 1–3 prefoveals; 0–1 subfoveal rows; lacunolabial present or absent; 9–14 chinshields in females and 8–13 in males; chinshields separated from infralabials by 7–10 scales in females and 7–9 in males; 4–6 gulars between chinshields and first ventral in both sexes; dorsal scale formula 35–39/33–37/24–26 and 34–38/31–37/24–25 in females and males, respectively; 197–211 ventrals in females and 198–204 in males; 46–53 subcaudals in both sexes; 8–17 rows of twice-divided subcaudals in females and 11–16 in males.

Color in preservative.—Dorsum of head creamish yellow or brown, with some sparse dark brown or black punctations. Black postocular stripe varying from 1.5–2.5 scale rows in width in both sexes. Dark brown or black rhomboidal dorsal blotches varying from 23–37 in males ($n = 10$) and 24–36 in females ($n = 8$). Blotches usually becoming narrower laterally, forming vertical bars. However, even when vertical bars are absent blotches are narrower laterally compared to *L. muta*.

Hemipenis.—General structure similar to *L. muta*; 81–98 spines in each lobe ($n = 3$).

Geographic distribution.—Atlantic slopes of Costa Rica, Panama, and southern Nicaragua (Campbell and Lamar, 2004; Fig. 4).

Lachesis melanocephala Solórzano and Cerdas, 1986

- 1986 *Lachesis muta melanocephala* Solórzano and Cerdas, J. Herpetol. 20:463–466
 1997 *Lachesis melanocephala*; Zamudio and Greene, Biol. J. Linn. Soc. 62:436

Diagnosis.—Distinguished from other species of *Lachesis* by having the dorsum of the head completely black.

Description.—Largest specimen a female 1800 mm SVL and 197 mm TL; body somewhat triangular in cross section; postnasals may contact first supralabial; internasals not distinct; four canthals; 11–12 intersupraoculars in both sexes; 29–31 interriectals in females and 28–29 in males; 8–9 supralabials

in both sexes; 13–14 infralabials in females and 12–13 in males; 0–2 prefoveals; 0 subfoveal scale rows; lacunolabial present or absent; 5–11 chinshields in females and 10–11 in males; chinshields separated from infralabials by 8–9 scales in females and 7–9 in males; 5–10 gulars between chinshields and first ventral in females, and 5–6 in males; dorsal scale formula 40–42/39–40/25 and 36–40/34–39/25–26 in females and males, respectively; 211–214 ventrals in females and 209–214 in males; 35–38 subcaudals in females and 39–40 in males; 13–15 rows of twice-divided subcaudals in females and 12–14 in males.

Color in preservative.—Dorsum of head completely black. Postocular stripe fused with the black dorsum of the head. Dark brown or black rhomboidal dorsal blotches vary from 24–35 in males ($n = 3$) and 26–37 in females ($n = 4$). Blotches similar to *L. stenophrys*.

Hemipenis.—General structure similar to *L. muta* and *L. stenophrys*; 91–96 spines on each lobe ($n = 4$).

Geographic distribution.—Restricted to Pacific coast of southeastern Costa Rica, especially at Osa Peninsula (Campbell and Lamar, 2004; Fig. 4).

RESUMO

O gênero *Lachesis* Daudin, 1803 foi dividido em seis grupos geográficos para se avaliar a posição taxonômica e as relações filogenéticas entre estes grupos. Foram usados caracteres relativos à morfologia externa e hemipênis. Resultados obtidos através das análises filogenética e estatística suportam o reconhecimento das espécies centro-americanas *L. melanocephala* e *L. stenophrys* como espécies distintas, enquanto a espécie sul-americana *L. muta* é reconhecida como monotípica, sem a divisão em subespécies. Tais resultados confirmam uma independência das linhagens da América Central em relação à América do Sul e são congruentes com outros estudos que utilizaram dados moleculares.

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APPENDIX I

Specimens Examined

Atropoides nummifer ($n = 4$).—Costa Rica: *Cartago*: Turrialba, Buena Vista: MZUCR 1502; *San Jose*: Tarrazu, San Marcos: MZUCR 112; *Puntarenas*: Coto Brus, Alturas de Coton: MZUCR 10877–78.

Bothriechis lateralis ($n = 3$).—Costa Rica: *Heredia*: Barva, P. N. Braulio Carrillo: MZUCR 9445; San Raphael, El Tirol: MZUCR 13257; *Alajuela*: Palmares, Palmares: MZUCR 12727.

Bothriechis nigroviridis ($n = 2$).—Costa Rica: *San Jose*: Dota, San Gerardo: MZUCR 11151; *Heredia*: Barva, P. N. Braulio Carrillo: MZUCR 9441.

Lachesis muta ($n = 229$).—Brazil: *Acre*: Rio Branco: MCPRS 2710; *Amapá*: Ferreira Gomes: IB 25404; Oiapoque: IB 24830; IB 13876; Rio Traçajuba: IB 25406; *Amazonas*: Presidente Figueiredo: MPEG 18845; MPEG 17589; MPEG 17554; MPEG 17763; MPEG 17522; IB 52391–93; IB 52406–11; IB 52924–26; IB 53440–41; IB 53542; IB 53423; IB 52707–09; Manaus: IB 52923; IB 43218; IB 45997; IB 43615; IB 40929; IB 43156; IB 41406; *Pará*: Altamira: IB 43149; Ananindeua: MPEG 16822; IB 46586; IB 48080; Apeú: MPEG 1203; Belém: MPEG 6359; IB 46558; IB 41296; IB 42952; IB 42656; IB 46123; Breves: IB 41521; Cametá: IB 2551; Colônia Nova (near Rio Gurupi-BR-316): MPEG 15058; Itaituba: IB 55687; Marabá: MPEG 10162; MPEG 16523; MPEG 16509;

Lachesis stenophrys ($n = 29$)*.—Costa Rica: *Alajuela*: São Miguel, Guapilito: MZUCR 12644; *Cartago*: Turrialba, Pavones: MZUCR 7146; *Heredia*: Sarapiquí, Porto Viejo (E. B. La Selva): MZUCR 3126; MZUCR 5804; *Limon*: Siquirres, Alto Guayacán: MZUCR 14079–80; MZUCR 13710; *Limon*: Talamanca: MZUCR 7412.

Lachesis melanocephala ($n = 7$)*.—Costa Rica: *Puntarenas*: Golfito, Pl. Blanca: MZUCR 1589.

* The specimens of *L. stenophrys* and *L. melanocephala* examined at Instituto Clodomiro Picado, San Jose, Costa Rica, have no collection numbers and, consequently, are not presented in the above list.