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Do Dietary Habits Predict Scale Counts in Snakes?

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Although studies on the biomechanical basis of feeding in snakes have revealed many links between dietary composition and feeding structures (e.g., Pough and Groves, 1983; Cundall, 1987), the strength and generality of such links have rarely been examined quantitatively. In this paper, I explore a simple ecomorphological question: can the type of prey eaten by a snake species be used to predict two aspects of its scale counts (the numbers of supralabial scales and dorsal scale rows)? The idea was first expressed more than 50 years ago by an innovative German scientist, Rudolf Mell (see Zhao and Adler, 1993). Based on his research in China, Mell noted that snake species eating larger prey (e.g., mammals rather than reptiles) had more supralabial scales and more midbody scale rows (Mell, 1929a,b). Mell suggested that an increased number of scale rows provides greater flexibility, enabling the skin between the scales to stretch over a large prey item.

More recent authors have repeated this suggestion. For example, Gans (1974) interpreted the increased number of scale rows on the neck of egg-eating snakes in this way. Pough and Groves (1983) interpreted high supralabial and midbody scale-row counts in viperid snakes (compared to colubrids) in the same fashion. Mell's original formulation was simplistic, however, and recent analyses of snake feeding provide more sophisticated views on these topics. For example, Greene (1983, 1997) has pointed out that prey items can be ''large'' in a number of ways; Mell's hypothesis relies upon prey being large in diameter rather than (for example) mass. Equally, it is a gross oversimplification to rate all mammals that are consumed by snakes as being larger (relative to the snake's size) than are all reptiles. Last, elasticity of the body could be enhanced by changing the structure of the skin, or increasing the amount of skin between scales, rather than by simply increasing the number of scale rows (Jayne, 1988). Given these issues, was Mell correct in suggesting an association between scale counts and dietary habits in Chinese snakes? And if so, how can this be true given the oversimplifications embedded in his assumptions?

I used Pope's (1935) tabulation of data on scale counts and prey types to test Mell's hypothesis on Chinese snakes. To examine the validity of the idea for a different assemblage, I also collated data on scale characters, body sizes and dietary composition for all terrestrial Australian snake species. Information on scale counts was obtained from publications by Wallach (1985), Storr et al. (1986), Barker and Barker (1994), O'Shea (1996), and Greer (1997). I recorded the mean (or modal, if no mean was reported) number of dorsal scale rows (the number of scales encircling the body, counted halfway between head and tail) and the number of supralabial scales (i.e., the scales along the top of the lip). Information on dietary composition and mean adult body sizes of snakes came from dissection and measurement of museum specimens (Shine, 1991, 1994). Diets were quantified in terms of the proportion of identifiable prey items falling into each major group (invertebrate, fish, amphibian, reptile, bird, mammal). Mean adult body size was calculated as the average of mean snout–vent lengths for adult males and adult females.

Three kinds of analysis were carried out on these data. First, I treated each species as a statistically independent unit and looked for the predicted correlations between the two types of scale characters (dorsal scale rows and supralabials) and between these traits and dietary composition (e.g., proportion of mammals in the diet). Second, because I found strong correlations between scale counts and body size (see below), I also compared dietary composition to size-corrected scale counts. To calculate size-corrected scores for each of the scale characters, I used residual scores from the general linear regressions of scale count versus mean body length. Thus, a species with a positive residual score for dorsal scale-rows would be one that had more scale-rows than would be expected for a species of that mean body size. Last, I combined the data with a putative phylogeny for these snakes (Fig. 1). This phylogenetic hypothesis was assembled by combining published suggestions about relationships among various subsets of the species involved (Kluge, 1991, 1993; Greer, 1997; Keogh et al., 2000; S. Keogh, pers. comm.). Hence, the resultant phylogeny (Fig. 1) is speculative and is not the result of any explicit phylogenetic analysis. Then I used this composite phylogeny to conduct a comparative analysis using independent contrasts (Purvis and Rambaut, 1995). This method overcomes the problem that many traits are highly conservative, so that correlations among traits may reflect phylogenetic inertia rather than functional relationship (Harvey and Pagel, 1991). Evolutionary changes in one variable (such as the proportional composition of the diet) within a clade can be compared to simultaneous changes in other traits (such as scalation) within the same clade. In the absence of reliable data on branch lengths within phylogenies, I assumed constant branch lengths (i.e., punctuated speciation events). Phylogenetically corrected relationships among variables were assessed by linear regression through the origin (Purvis and Rambaut, 1995). Statistical analyses were performed using CAIC (Purvis and Rambault, 1995) and Statview 5 on an Apple Macintosh G4 computer. Prior to analysis, data were tested for conformity with assumptions of normality required for tests.

A one-factor ANOVA on Pope's 1935 data (his table LX) with prey type as the factor and number of scale rows as the dependent variable, confirms Mell's (1929a,b) assertion that dietary habits correlate with scale counts within the Chinese snake fauna ($F_{5,112} = 3.88$, $P < 0.003$). Posthoc (Fishers' PLSD) tests show that species that eat mammals have significantly more

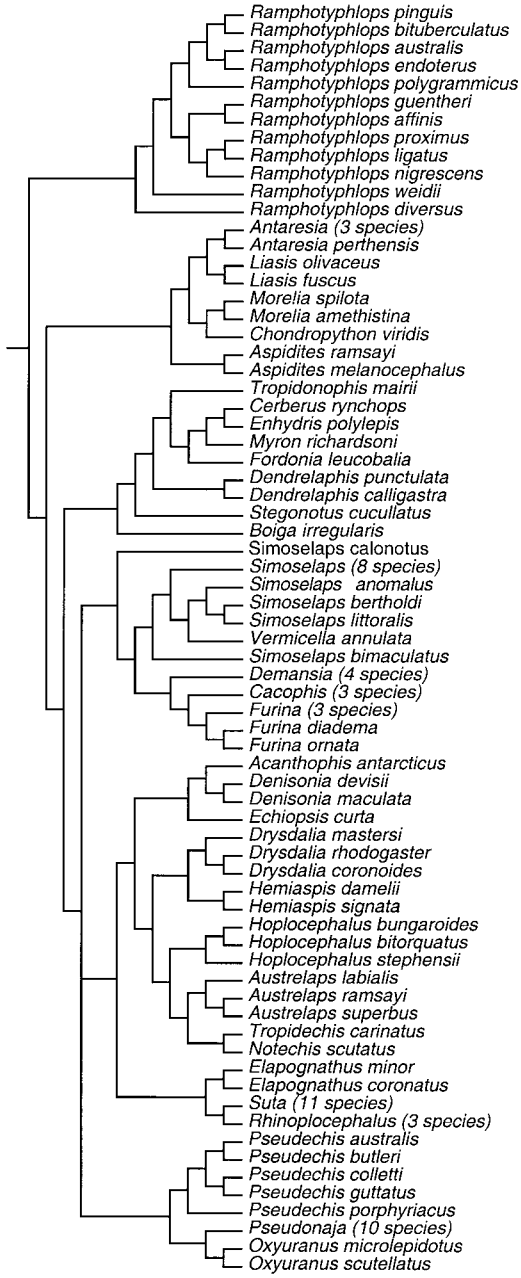

FIG. 1. Phylogenetic hypothesis for Australian snakes, as used in the present study. See text for list of sources for this phylogeny.

midbody scale rows than those that eat reptiles, frogs or invertebrates ($P < 0.05$ in each case).

Data were obtained for 108 Australian species, comprising 11 pythonids, nine colubrids, 76 elapids and 12 typhlopids. Mean adult snout–vent length ranged from 17–212 cm, midbody scale rows from 13–67, and supralabial scale counts from 4–14. The proportion of the diet composed of various prey types also varied widely (from 0–100% for each of fishes, reptiles, and mammals; from 0–97% for amphibians; 0–36% for birds).

Overall, my analyses for the Australian taxa revealed the same general pattern as noted by Mell for Chinese snakes but cast substantial doubt on the mechanism that he suggested to explain this pattern. Table 1 summarizes results from statistical tests of three predictions from Mell's hypothesis.

Number of Midbody Dorsal Scale-Rows Is Positively Correlated with Number of Supralabial Scales.— As predicted, species with high numbers of midbody dorsal scale rows also had many supralabial scales (Fig. 2, Table 1). However, both of the scale counts also showed strong positive correlations with mean adult body size. These relationships differed among families. For example, at any given body size, pythons have more supralabial scales than elapids, and colubrids are intermediate in this respect (Fig. 3). The correlation between dorsal and labial counts remained high even after the effects of body size were removed (Table 1). That is, the trend for species with high numbers of scale-rows to also have many supralabial scales is not an indirect consequence of body size.

Comparative analysis further supported the notion of a functional relationship between scale numbers around the body versus along the upper lip. Increases in midbody scale rows during phylogeny were consistently associated with increases in supralabial counts (Table 1). After removing body-size effects from the comparative analysis, shifts in relative numbers of midbody dorsal scale-rows (i.e., residual scores) were associated with shifts in relative numbers of supralabial scales (i.e., residual scores; $N = 70$, $r = 0.65$, $P < 0.0001$).

Species that Eat Larger Prey Types Have More Dorsal Scale Rows.—As in the Chinese fauna, Australian snake species that feed on birds and mammals tend to have more dorsal scale rows than do other species (Table 1; Fig. 4). When the effects of body size on scale counts are removed, however, none of the probability values for these analyses attain the conventional level of significance ($P < 0.05$) after Bonferroni corrections are applied (Table 1).

Phylogenetically based analysis revealed a consistent association between dorsal scale rows and the proportion of mammalian prey in the diet but not for the other prey groups (Table 1). Using size-corrected scores (i.e., residual values from scale counts versus SVL) greatly weakened the apparent link between scalation and diet: no dietary proportions were significantly correlated with size-corrected scale counts ($P > 0.10$ in all cases).

Species that Eat Larger Prey Types Have More Supralabial Scales.—Patterns for this variable are similar to those for dorsal scale rows (above), as might be expected from the high correlation between the two traits (Fig. 2). Species that feed on birds and mammals have more supralabial scales than do species that feed on ectotherms (Fig. 4), but this pattern disappears after body-size effects are removed from the analysis (Table 1). Phylogenetic shifts in supralabial counts were correlated (albeit weakly) with shifts in the proportion of mammalian prey (Table 1), but this effect disappeared when size-corrected values were used in the analysis (all $P > 0.10$).

TABLE 1. Relationsihps among scale characters and dietary composition in Australian snakes. The table provides results from linear regression analysis of three types of data. First, values for each species were used as independent datapoints (''raw data''). Then, because both types of scale counts were highly correlated with overall body size, the effects of size were removed by calculating residual scores from the general linear regressions of the scale trait against mean adult snout–vent length (''size-corrected data''). Third, comparative analysis using independent contrasts was used to remove the effects of phylogenetic conservatism. Sample size was 108 for the first two types of tests and 70 for the comparative analysis. The table shows the correlation coefficient (r) and its associated probability (P). **Boldface** shows results that are statistically significant ($P < 0.05$) after Bonferroni correction.

Traits being compared	Raw data		Size-corrected data		Comparative analysis	
	r	P	r	P	r	P
Dorsal scale rows vs supralabial scales	**0.82**	**0.0001**	**0.71**	**0.0001**	**0.70**	**0.0001**
Dorsal scale rows vs. % fishes	0.03	0.77	0.09	0.35	0.09	0.45
Dorsal scale rows vs % frogs	−0.15	0.13	0.09	0.35	−0.19	0.11
Dorsal scale rows vs % reptiles	**−0.28**	**0.0004**	−0.22	0.026	−0.04	0.75
Dorsal scale rows vs % birds	**0.43**	**0.0001**	0.21	0.028	0.14	0.23
Dorsal scale rows vs % mammals	**0.49**	**0.0001**	−0.02	0.85	**0.31**	**0.008**
Supralabial scales vs % fishes	0.13	0.18	0.22	0.03	0.04	0.77
Supralabial scales vs % frogs	0.01	0.92	−0.01	0.93	−0.07	0.55
Supralabial scales vs % reptiles	−0.05	0.60	0.16	0.11	0.05	0.70
Supralabial scales vs % birds	**0.41**	**0.0001**	0.20	0.04	0.13	0.30
Supralabial scales vs % mammals	**0.46**	**0.0001**	−0.03	0.79	**0.28**	**0.02**

The end result of these analyses is that three traits (body size, dietary habits, and scale counts) are significantly intercorrelated. Although it is difficult to distinguish direct from indirect effects within such constellations of traits, partial correlation offers a partial solution. I used this technique to evaluate the correlation between dietary habits and one variable (such as scale-row number) when another factor (such as body size) was held constant. Two sets of these analyses were conducted, one on the raw data and one on phylogenetic contrasts. In both cases, partial-correlation analyses suggested that dietary habits were more highly associated with body-size than with scale counts. For the raw data, the proportion of the diet composed of mammals was significantly correlated with mean adult SVL when either scale-row numbers or supralabial counts were held constant ($N = 108$, $r = 0.63, 0.71$, respectively, $P < 0.001$ in both cases) but neither of the scale characters was correlated with dietary habits if SVL was held constant ($N = 108$, $r = -0.03, 0.05$, respectively, $P > 0.50$). No other partial correlations between dietary habits and morphological traits were statistically significant ($P < 0.05$) after Bonferroni correction for multiple tests. The comparative analysis (independent contrasts) provided an almost identical result. The proportion of mammals in the diet was correlated with mean adult SVL when either of the scale characters was held constant ($N = 70$, $r = 0.56, 0.55$, respectively, $P < 0.001$ in both cases) but not with either of the scale counts when SVL was held constant ($N = 108$, $r = -0.13, -0.04$, respectively, $P > 0.50$). These results suggest that the correlations between scale counts and diet are secondary consequences of body-size effects on both scalation and diet.

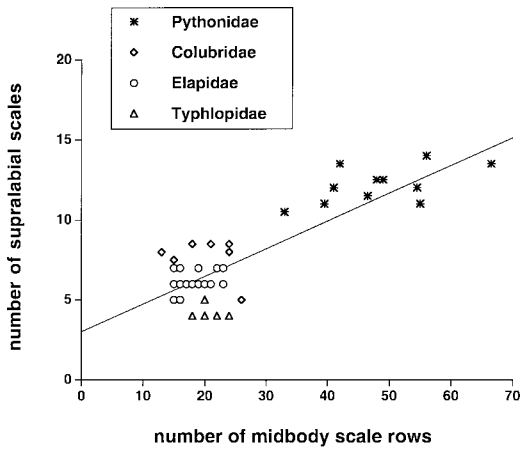

FIG. 2. The relationship between the numbers of dorsal scale rows and supralabial scale count in an interspecific comparison among Australian snakes. Each point represents one species. See Table 1 for results of a test of the statistical significance of this relationship.

If indeed we could find strong links between ecological traits (such as dietary composition) and morphological features (such as the numbers of dorsal scale-rows), such associations would be of considerable value. For example, we have quantitative information on dietary composition for only a small proportion of snake species. Unfortunately, my study is not encouraging with respect to inferring diet from scalation (or vice versa). There are at least three processes that might generate a correlation between dietary composition and scale counts in snakes. First, interspecific divergences in dietary habits (especially, relative prey size) might have imposed selection on scale-row numbers as envisaged by Mell. Second, the

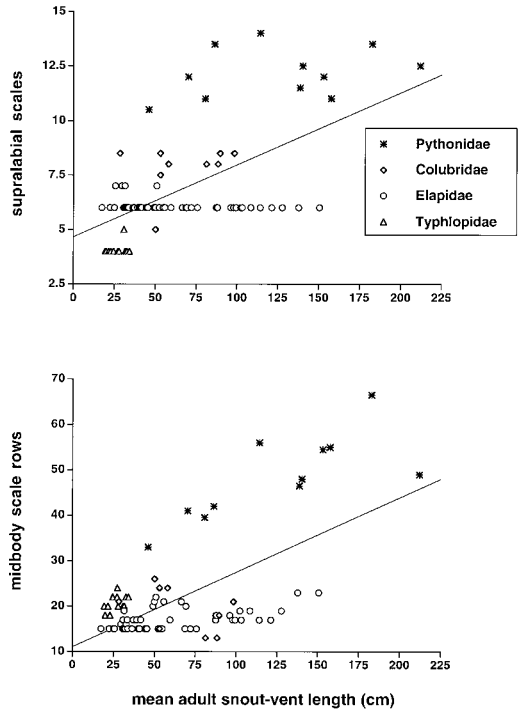

FIG. 3. The relationship between body size (mean adult snout–vent length) and scale counts in Australian snakes. Lower graph shows data for the number of dorsal scale rows, and upper graph shows the data for the number of supralabial scales. Each point represents one species. Linear regression for these datasets shows: dorsal scale rows, $N = 108$, $r = 0.65$, $P < 0.0001$; supralabial scales, $N = 108$, $r = 0.62$, $P < 0.0001$.

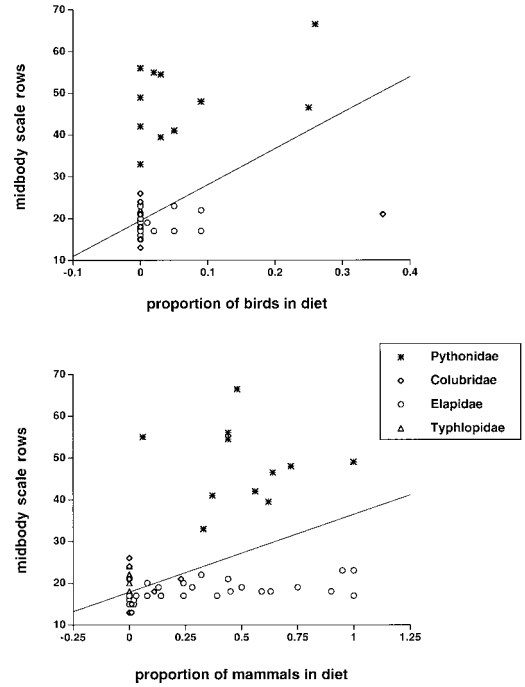

FIG. 4. Scale characteristics of Australian snakes in relation to dietary composition. Species that consume a higher proportion of birds and mammals tend to have more dorsal scale rows and more supralabial scales. See Table 1 for tests of the statistical significance of these relationships.

correlation between scale counts and dietary composition might be an artifact of phylogenetic inertia, because some lineages of snakes have low values for all three traits (supralabials, dorsal scale rows, and proportion of the diet composed of endotherms) whereas other lineages have high values for all three traits. This circumstance might have arisen early in snake phylogeny, and require no adaptive explanation in terms of selective forces during the radiation of species within lineages. In practice, phylogenetic shifts in the degree of reliance on mammalian prey were consistently accompanied by shifts in scale counts (Table 1), suggesting a functional association between the traits. Nonetheless, phylogenetic conservatism undoubtedly amplifies the strength of these correlations (Table 1, Fig. 4). Last, correlation does not imply causation. A third factor (mean adult body size) is known to correlate both with dietary habits (e.g., Fitch, 1960; Arnold, 1993; Shine, 1994) and with scale counts (e.g., Klauber, 1956; Lindell, 1994). Thus, a shift in food habits toward larger prey could favor the evolution of larger body size, which in turn could favor an increase in scale counts (because larger snakes have more scales: see Fig. 3). In keeping with this hypothesis, correlations between scale counts and dietary habits generally disappeared when body-size effects were removed from the analysis (Table 1).

In summary, Mell was probably correct in recognizing a general association between scale counts and diets in snakes, but wrong about its cause. To fully understand the pattern identified by Mell 50 years ago, we will need to gather data on traits that relate much more closely to the actual selective pressures and functional challenges involved in prey ingestion. For example, many researchers in snake ecology still report only the species and mass of prey items, despite heartfelt appeals to take more extensive data (e.g., Greene 1997). Maximum prey diameter is simple to measure (even with partially digested prey) and is likely to be a better measure of the physical difficulty of ingesting the item than is prey mass. Until we have extensive datasets on such variables, many of the ideas espoused by visionaries like Rudolf Mell will remain untested.

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Testing for Equal Catchability of *Triturus* Newts by Dip Netting

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Testing the assumption of equal catchability is fundamental to understanding amphibian populations, modeling population dynamics, and testing hypotheses concerning life-history traits. Unequal catchability forms an important source of potential error in quantitative surveying, affecting the estimation of population parameters such as population size, in particular when the capture-recapture method is used (Bohlin and Sundström, 1977; Carothers, 1979; Pollock et al., 1990). Nonrepresentative sampling has been reported for many animal species in a wide range of taxa, and it has been questioned whether populations consisting of animals with equal catchability actually exist (Carothers, 1973). In amphibian studies, sampling bias has been mostly ignored, with some notable exceptions (Gelder and Rijsdijk, 1987; Shirose and Brooks, 1995; Wood et al., 1998; Wilson and Pearman, 2000).

The techniques available for the study of amphibians include some sophisticated approaches (Heyer et al., 1994; Mölle and Kupfer, 1998). However, the sampling of many different habitats will be facilitated by the use of simple, small, and inexpensive tools, such as the traditional dip net. Amphibians that breed over long periods in lentic water and those with larval stages of long duration are especially amenable to netting. Palearctic newts of the genus *Triturus* fall into this cat-

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egory, and netting is an easy and reliable method to indicate their presence. It is not known, however, whether netting also yields reliable quantitative results. I here test the hypothesis that *Triturus*-samples obtained by dip netting are representative for the entire aquatic population. A practical evaluation of popular sampling techniques for some widespread European species is provided by Griffiths and Raper (1994).

Research was carried out in 10 ponds in the ''département'' Pas-de-Calais in northwestern France and in 16 ponds in the département Mayenne in western France over the period 1975–2001. The ponds were small, with a surface area not exceeding 200 m^2. The dip net used was a triangular metal frame with sides of 32 cm mounted to an aluminum stick of 150 cm length. The netting material was lace curtain of 4 × 4 mm mesh. Netting sessions lasted up to 90 min, depending on pond size, density of aquatic vegetation, and capture results. In an attempt to achieve a constant sampling effort, equal attention was paid to different sections of the pond. Captured adult newts were identified to sex and species, marked by clipping one toe, and released at the end of the session. Clipped toes regenerate, but marks were clearly distinguishable over the duration of the experiments, and different marks were used between years. The five *Triturus*-species observed were the small-bodied *Triturus helveticus* and *Triturus vulgaris*, the medium-sized *Triturus alpestris* and the large-bodied *Triturus cristatus* and *Triturus marmoratus*. The rare hybrids *T. helveticus* × *T. vulgaris* and *T. cristatus* × *T. marmoratus* were not included in analyses (Arntzen and Wallis, 1991; Arntzen et al., 1998). Ponds were visited from three to six times within a two-month period. Group membership (sex, species, or size category) and marking status (first encounter vs recapture) were compared in contingency tables with data pooled over sessions. All experiments involved 50 or more individuals and five or more recaptures. Because no individual marks or date-marks had been applied, I did not follow the standard procedure of separate analysis of data per recapture session (Seber, 1982:160). Instead, discrepancies between observed frequencies across groups were tested with *G*-tests of independence, with Williams' correction for small sample size (Sokal and Rohlf, 1981); a significant result indicated greater catchability for one group as opposed to the other. Interpretations were subjected to Bonferroni correction to adjust for multiple tests (Holm, 1979). In some cases, the power of the tests was low, so I also analyzed results across groups with the parameter G' that measures the degree to which sampling was nonrepresentative. This newly designed G' equals G but is positive or negative depending on the group with the highest recapture frequency. I used the one sample *t*-test to test the observed distribution of G' against zero and ANOVA to compare G' among groups. In addition to my own data, I analyzed two published datasets for comparison (Hagström, 1979; Kletecki, 1995).

One hundred eight tests for representative sampling were made between males and females of five species. Additionally, 74 tests involved species of similar size and 53 tests involved species of dissimilar size. The average sample size was 264 per experiment (range 50–1666). The average recapture proportion was 29%. The numbers of first encounters and recaptures did not show significant departures from expectations of random sampling when the sexes were compared (Bonferroni corrected, Table 1). Similar-sized species with data for sexes pooled showed one significant result ($P < 0.01$) with higher recapture numbers for *T. helveticus* than for *T. vulgaris*. Comparing species from different size categories yielded two significant results, with relatively more recaptures among the large-bodied than among the small-bodied newts (*T. marmoratus* over *T. helveticus*, $P < 0.05$; pooled *T. cristatus* and *T. marmoratus* over pooled *T. helveticus* and *T. vulgaris*, $P < 0.01$).

Relative recapture numbers as expressed by G' did not differ significantly between the sexes, species, or size classes, with the exception of small-bodied newts (pooled *T. helveticus* and *T. vulgaris*) that were recaptured more frequently than the medium-sized *T. alpestris* ($P < 0.05$). Significant differences in relative recapture numbers between the species were not observed (ANOVA, $F_{4,100} = 1.96$, $P > 0.10$).

Published data on newts from Sweden (Hagström, 1979) and Croatia (Kletecki, 1995) yielded 18 comparisons between sexes and 10 comparisons between species. Sample size ranged from 93–2517, and the average recapture proportion was 36%. Differences in catchability were not found between the sexes. Species comparisons yielded five significant results (Bonferroni corrected, Table 2). The recapture frequency in Sweden was higher for *T. cristatus* than for *T. vulgaris* in two cases of four ($P < 0.05$ and $P < 0.01$). Conversely, one case was observed in Croatia of a higher recapture frequency for *T. vulgaris* than for *T. carnifex* (a species closely related to *T. cristatus*; $P < 0.001$), with *T. alpestris* taking an intermediate position ($P < 0.001$ relative to *T. vulgaris*; $P < 0.05$ relative to *T. carnifex*).

I studied the catchability of five *Triturus* species in syntopic populations. I found no indications that netting yielded different results for males and females. Comparisons for species and size groups indicated some sampling bias, but the results were inconsistent. The hypothesis that small-bodied species are easier to catch than large-bodied species (Cooke and Frazer, 1976; Beebee, 1990) was not supported. This result is consistent with the data from Sweden (Hagström, 1979) and contradict data from Croatia (Kletecki, 1995).

The main assumptions underlying the analysis were (1) the absence of marking effects, (2) no differential mortality for the duration of the experiment, and (3) similar pond immigration and emigration timetables across groups. First, marking has negligible effects on survival and body condition (Arntzen et al., 1999), and I consider it unlikely that marking by toe clipping would have caused a substantial sampling bias. Second, *Triturus* newts are long-lived animals with negligible mortality over a time span such as considered here (e.g., for life-history data on *T. cristatus*, see Francillon-Vieillot et al., 1990; Arntzen and Teunis, 1993). Third, the largest documented phenological difference within the genus *Triturus* is that between *T. marmoratus* with a short (approximately 2-month) aquatic period and *T. cristatus* with a long (approximately 5-month) aquatic period (Bouton, 1986; Arntzen and Wallis, 1999; Arntzen, 2002). This could

TABLE 1. Data summary and tests for discrepancy between the number of ''first encounter'' and recaptured *Triturus*-newts between sexes, species, and size classes. Tests are *G*-test for independence and *G*′ based *t*-tests (details see text). All experiments involved 50 or more individuals. * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$, before (a) and after (b) Bonferroni correction for multiple tests. N.S., not significant; N.A., not applicable because of small number of experiments.

Groups analyzed	Number of		Sample size average (range)	Recapture proportion average	G-test of independence		*G*′ test
	Experiments	Ponds			(a)	(b)	
Males and females							
Small bodied species							
T. helveticus	30	9	448 (55–1524)	26%	1**		N.S.
T. vulgaris	9	3	142 (50–421)	25%			N.S.
Medium sized species							
T. alpestris	8	4	77 (53–111)	18%			N.S.
Large bodied species							
T. cristatus	13	8	96 (53–155)	35%	2*		N.S.
T. marmoratus	48	4	126 (59–249)	28%			N.S.
Total	108	17	210	27%	3	0	N.S.
Species of similar size							
T. helveticus–T. vulgaris	26	7	472 (78–1125)	25%	2*, 1***	1**	N.S.
T. cristatus–T. marmoratus	48	9	123 (50–314)	31%	2*, 1**		N.S.
Total	74	15	246	29%	6	1	N.S.
Species of dissimilar size							
Small–medium	17	7	392 (81–1176)	22%	3*, 1**		*
Small–large	33	7	431 (80–1666)	26%	2*, 1**, 2***	1*, 1**	N.S.
Medium–large	3	2	100 (75–133)	14%	1*		N.A.
Total	53	10	400	24%	10	2	N.S.

TABLE 2. Data summary and tests for discrepancy between the number of ''first encounter'' and recaptured *Triturus*-newts between sexes, species, and size classes. Data are from Hagström (1979) and Kletecki (1995). * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.01$, before (a) and after (b) Bonferroni correction for multiple tests.

Groups analyzed	Number of		Sample size average (range)	Recapture proportion average	G-test of independence	
	Experiments	Ponds			(a)	(b)
Males and females						
Sweden						
T. vulgaris	8	2	151 (115–231)	43%		
T. cristatus	4	1	254 (133–336)	30%		
Croatia						
T. alpestris	2	2	1221 (193–2249)	38%		
T. carnifex	2	2	299 (218–379)	34%		
T. vulgaris	2	2	181 (93–268)	45%		
Total	18	4	312	39%	0	0
Species of dissimilar size						
Sweden						
T. cristatus–T. vulgaris	4	1	303 (183–451)	20%	1**, 1***	1*, 1**
Croatia						
T. alpestris–T. vulgaris	2	2	1402 (286–2517)	38%	1***	1***
T. carnifex–T. vulgaris	2	2	479 (472–486)	39%	1***	1***
T. alpestris–T. carnifex	2	2	1520 (572–2467)	35%	1**	1*
Total	10	3	801	30%	5	5

introduce a numerical bias through marked newts leaving and unmarked newts entering the pond. However, experiments were restricted to a 2-month period mostly coinciding with the peak of the newt breeding season in April and May, and excluded the peaks of the migratory season. The average difference between the first and the last capture was 17 days, indicating that the populations could be considered ''closed'' from a capture-mark-recapture point of view.

A marked niche differentiation could affect catchability. Studies with (fixed) bottle-traps did not show consistent differences between the aquatic niches of similar sized species (Griffiths, 1987; Jehle et al. 2000). Conversely, small- and large-bodied species have different aquatic niches, with *T. vulgaris* more or less evenly distributed across the pond and *T. cristatus* preferentially occupying the bottom of the water column and the middle of the pond (Griffiths and Mylotte, 1987). *Triturus helveticus* occurred at shallower sections of the pond than *T. cristatus* and *T. marmoratus* (Jehle et al., 2000). The several substantiated cases of dip net sampling bias may reflect niche differentiation of the species involved and were probably brought about by particular pond characteristics, such as deep water, aquatic vegetation, and obstacles (e.g., fallen trees, barbed wire) rendering some sections of the pond more difficult to sample than others.

I conclude that sampling by netting generally produces data that are representative for *Triturus* populations as a whole and that the technique is appropriate for quantitative surveying, provided sampling takes place in all sections of the pond. A disadvantage of netting—and a cautionary note—is that the dip net has to be thoroughly cleaned in between pond visits not to transport invasive species, including disease organisms (e.g., Bosch et al., 2001).

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Seasonal Abundance of Hatchlings and Gravid Females of *Sphaerodactylus nicholsi* in Cabo Rojo, Puerto Rico

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Sphaerodactylus is a genus of voiceless, Neotropical geckos that is believed to include the smallest amniotes (MacLean, 1985; Rivero, 1998). Despite their small size, sphaerodactylids appear to possess no particular physiological adaptations to control the rate of evaporative water loss (EWL). MacLean (1985) showed that a very small species, *Sphaerodactylus parthenopion*, and four populations of a larger species, *Sphaerodactylus macrolepis*, fall on the projected extension of the log EWL/log body mass regression calculated by Mautz (1982) for lizards from mesic and semiarid environments. Because of their extremely small size and high surface-to-mass ratio, sphaerodactylids are subject to high EWL and may rely on microhabitat selection rather than physiological adaptation to survive in the hot dry environments they often inhabit. Within species the smallest and potentially most vulnerable individuals are the hatchlings. Reduction of water stress beyond that provided by habitat selection alone could be accomplished for these smallest individuals if reproduction were timed to produce hatchlings during the colder months when days are shorter and solar radiation is less intense.

Although the systematics and evolution of *Sphaerodactylus* have been actively studied (e.g., Thomas and Schwartz, 1966a,b; Shreve, 1968; Thomas, 1975; MacLean and Holt, 1979; Murphy et al., 1984; Hass, 1991), ecological, behavioral, and physiological information is relatively scarce (Gaa-Ojeda, 1983; MacLean, 1985; Leuck et al., 1990; Meier and Noble, 1990). *Sphaerodactylus nicholsi* and *Sphaerodactylus townsendi* were formerly considered subspecies of the same taxon. Analysis of genetic data led to their separation at the species level (Murphy et al., 1984). They remain, however, the most similar species pair among the West Indian sphaerodactylids analyzed electrophoretically by Hass (1991). These two species and *S. parthenopion* represent the xeric forest ecomorph of Thomas et al. (1992).

Sphaerodactylus nicholsi is the smallest of the endemic sphaerodactylids in Puerto Rico (about 20 mm in snout–vent length, SVL, López-Ortiz, 1999, compared to 18 mm SVL for *S. parthenopion*, Thomas, 1965) and is perhaps the most easily distinguished (Rivero, 1998). This species is sexually monomorphic and is distributed discontinuously along the coast of Puerto Rico in the north-central, western, and south-central regions (Schwartz and Henderson, 1991). It is considered diurnal, although it may not be exclusively so, and in southwestern Puerto Rico is found in dense leaf litter under evergreen tree species in open semideciduous dry forest (Rivero, 1998; López-Ortiz, 1999).

Gaa-Ojeda (1983), in one of the rare ecological studies of the genus, described aspects of life history of *S. townsendi* and *S. macrolepis* in coastal areas of eastern Puerto Rico. Although she did not observe a significant correlation between annual climate variation and patterns of reproduction in *S. townsendi*, Gaa-Ojeda suggested that the combination of low temperature and low rainfall, characteristic of December and January, induced reproductive activity in females and allowed incubation during warmer, wetter months. She did not discuss the impact of thermal or hydric stress on hatchlings, but the cyclicity in reproduction that she found resulted in highest abundance of hatchlings and juveniles during September through November when the days grow shorter.

As part of a nondestructive study of the temporal and spatial distribution of *S. nicholsi* in relation to habitat variables in southwestern Puerto Rico, we inferred the pattern of seasonality of reproduction from counts of hatchlings and gravid females in monthly population surveys. We compared the pattern in *S. nicholsi*

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with the pattern reported for *S. townsendi* in northeastern Puerto Rico (Gaa-Ojeda, 1983) and examined these data for correlations with climatic variables. Because of the potential vulnerability of hatchlings to evaporative water loss, we hypothesized that any cyclicity of reproduction should result in maximum relative abundance of hatchling *S. nicholsi* during months of low heat stress. We also considered food availability and incubation environments as additional factors that could influence timing of reproductive cycles.

Sphaerodactylus nicholsi is patchily distributed in litter beneath trees on the Cabo Rojo unit of the Caribbean Islands National Wildlife Refuge (67°10′7.2″N 17°58′1.3″W; López-Ortiz, 1999). The refuge is characterized by open secondary forest, grassland, and brush habitats. For capture-recapture studies, we selected the three highest density aggregations from 33 aggregations found in a random sample of 60 quadrats (2500 m^2/quadrat). Aggregations were delimited by the dense shade from a single large tree or a cluster of trees. The area occupied by an aggregation was roughly circular with a diameter on the order of 4 m. During the last five days of each month, from June 1997 to May 1998, approximately 20 geckos/day/aggregation were captured from the three aggregations, SVL was measured, and a day-specific paint mark was applied to the abdomen. Paint marks did not persist reliably from month to month. They were used to assure that an individual was counted only once per month and to demonstrate that the capture and release did not cause declines in population numbers of these lizards within months (López-Ortiz, 1999). We believe that many lizards were captured in more than one month, but we treat measurements as independent over months. Reproductive data were gathered when the lizards were measured. Although males and females could not be distinguished, gravid females were identified by observing the oviductal egg through the skin on the abdomen of the gecko. The lizards we term ''hatchlings'' were those with the smallest SVL (8–12 mm) and with dorsal colors that varied from black to dark brown. All lacked the light brown body and the yellow dewlap and tail of the adults. The ventral colors of hatchlings varied from light to dark gray; ventral colors of adults were yellower. We call them hatchlings because they were even smaller than the hatchlings defined by Gaa-Ojeda (1983) for *S. townsendi* on the basis of dissections (12–13 mm SVL); we prefer hatchling to juvenile because it emphasizes their minute size. The lizards we term ''nongravid adults'' were larger and had adult coloration but no egg. We have not demonstrated by dissection that the change in color is associated with onset of sexual maturity, so some of our nongravid adults may not have been mature. Each gecko was processed in an average of one minute and then released at the center of its aggregation area.

The population in Aggregation 3 crashed in January 1998. The crash occurred in association with the immigration of three *Ameiva exsul* (Teiidae) in November 1997, with two more *A. exsul* arriving in January. This species is known to eat sphaerodactylids, and it has been shown to depress abundance of prey species in the leaf litter (Lewis, 1989). The five teiids actively foraged in the leaf litter of aggregation three and established burrows. Prior to arrival of the teiids the population had remained stable for five months despite monthly capture-recapture sampling. Therefore, no data from Aggregation 3 are included in the analyses for the months January through May. The population densities of the other two aggregations, without teiids, remained stable throughout the year of the study.

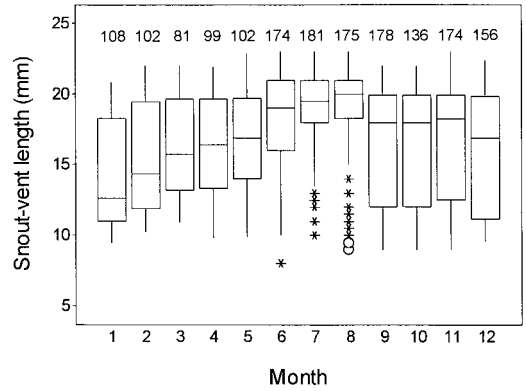

FIG. 1. Snout–vent lengths (SVL) of *Sphaerodactylus nicholsi* from three neighboring high density aggregations in Cabo Rojo, Puerto Rico. The horizontal line in the middle of each box and whisker plot gives median SVL; the box gives the inner quartiles; the vertical lines end on datapoints and can be no longer than 1.5 times the length of the box; the circles and pluses give outliers, so complete ranges are defined; the numbers at the head of each plot give sample size. The figure is plotted with the abscissa starting in January; the sample year started in June.

Seventeen-year average rainfall and temperature data were supplied by Joseph Schwagerl from the USFWS/NWR staff. The 1997–1998 temperature and insolation data for the Refuge, covering the months of the study, were provided by Jorge González (Department of Mechanical Engineering, University of Puerto Rico at Mayagüez). The average global insolation in this area for a 20-month period in 1980–1981, a sample of historical data, was obtained from López and Soderstrom (1983). We obtained data on day length from the Nautical Almanac for the Year 1987 (US Naval Observatory, 1986). Reproductive data were compared with these climatic data. Statistics were calculated using SPSS (SPSS Inc., Chicago, IL, 1999) and Statistix for Windows (Analytical Software, Tallahassee, FL, 1996).

Snout–vent length of geckos did not vary significantly among the three areas (one-way ANOVA: $F_{2,30} = 1.585$; $P = 0.222$), so measurements from the three areas were pooled for each month. SVL did vary significantly among months (Kruskal-Wallis: $\chi^2 = 190.02$; df = 11; $P < 0.001$). Median SVL was greatest in June, July, and August, and smallest in January (Fig. 1). Lizards of all sizes were present throughout the year, but median SVL increased from January to the peak in August. Over all months SVL averaged 15.67 mm (SE = 0.387, $N = 12$ months) with monthly average SVL as observations (total $N = 1666$ for all 12 months). Individual SVL ranged from 8.0–23.0 mm.

Gravid females were often found hiding in the bases of bushes or grass clumps (e.g., *Croton* sp. and *Panicum*

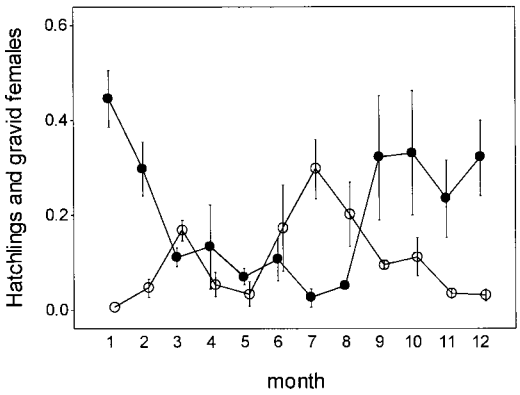

FIG. 2. Proportional abundance of hatchlings (closed circles) and gravid females (open circles) of *Sphaerodactylus nicholsi* from three neighboring high density aggregations in Cabo Rojo, Puerto Rico. Vertical lines give standard errors. The proportional abundance of nongravid adults is obtained by subtracting the sum of values for hatchlings and gravid females for each month from 1.0. The numbers in each reproductive category are obtained by multiplying the proportions by the sample sizes in Figure 1.

maximum). The percentage of gravid females did not vary significantly among areas (Kruskal-Wallis: $\chi^2 = 0.921$; df = 2; $P = 0.631$), so measurements from the three areas were pooled. The percentage of gravid females averaged 10.16 over all 12 months (SE = 2.57, $N = 12$ months). The relative abundance of gravid females varied significantly among months (Fig. 2; Kruskal-Wallis: $\chi^2 = 138.773$; df = 11; $P < 0.001$). No gravid females were observed during January, while July had the highest percentage; there was a secondary peak in March. The SVL of gravid females averaged 20.29 mm (SE = 0.135, $N = 11$ months) with monthly average SVL used as observations to assure independence (total $N = 185$ gravid females for the 11 months). Individual SVL for gravid females ranged from 15.7 mm to 23.0 mm. This is smaller than the size range for adult female *S. townsendi* (21.5–30.0 mm) given by Gaa-Ojeda (1983).

The percentage of hatchlings did not vary significantly among areas (Kruskal-Wallis: $\chi^2 = 2.766$; df = 2; $P = 0.251$), so measurements from the three areas were pooled. The overall percentage of hatchlings (across all 12 months) averaged 20.57 ± 8.92% (SE = 4.06, $N = 12$). The percentage of hatchlings varied significantly among months (Fig. 2; Kruskal-Wallis: $\chi^2 = 185.926$; df = 11; $P < 0.001$). July and August had the lowest percentage of hatchlings while January had the highest; there was a secondary peak in September. The average SVL for hatchlings was 11.04 mm (SE = 0.096; $N = 12$ months) with monthly average SVL as observations (total $N = 336$ hatchlings for the 12 months). Individual SVL of hatchlings ranged from 8.0 to 12.0 mm.

Hatchling SVL varied significantly across months (one-way ANOVA applied to ranks: $F_{11,324} = 2.01$; $P = 0.027$) but no pairwise comparisons of months were significantly different. Similarly, size of gravid females varied significantly across months (one-way ANOVA applied to ranks: $F_{10,174} = 2.77$; $P = 0.003$) with no significant pairwise differences. Nongravid adults, however, increased in size from January to August (one-way ANOVA applied to ranks: $F_{11,1133} = 7.00$; $P < 0.001$). SVL was larger in August than in January, February, March, April, and May, whereas SVL was smaller in March than in June, July, August, September, November, and December (Analytical Software, Tallahassee, FL, 1996; following Daniel, 1990). We attribute the increase in SVL from January to August to the increase in body size of nongravid adults moderated by the transformation of hatchlings into the adult color class.

The monthly percent of hatchlings in the sample was negatively correlated with the historical average monthly insolation (Spearman's rho: $r_s = -0.739$; $N = 12$; $P = 0.006$), average monthly insolation in the year of the study ($r_s = -0.623$; $P = 0.030$), and average monthly day length ($r_s = -0.755$; $P < 0.010$). However, relative abundance of hatchlings was uncorrelated with rainfall in the year of the study ($r_s = 0.0350$; $P = 0.914$) and uncorrelated with average rainfall ($r_s = -0.0909$; $P = 0.779$).

The monthly percentage of gravid females in the sample was positively correlated with the historical average monthly insolation ($r_s = 0.599$; $N = 12$; $P = 0.040$), average monthly temperatures ($r_s = 0.585$; $P = 0.046$), and average monthly day length ($r_s = 0.713$; $P = 0.009$). Relative abundance of gravid females was uncorrelated with rainfall in the year of the study ($r_s = -0.0909$; $P = 0.779$) and uncorrelated with average rainfall ($r_s = 0.0280$; $P = 0.931$).

The negative correlations over months of percentage of hatchlings with both percentage of gravid females ($r_s = -0.671$; $P = 0.017$) and percentage of nongravid adults ($r_s = -0.7343$; $P = 0.007$) were significant. When hatchlings were lagged by six months, the correlation between percentage of hatchlings and percentage of gravid females became significantly positive ($r_s = 0.769$; $P < 0.005$), whereas the correlation between percentage of hatchlings and percentage of nongravid adults became nonsignificant ($r_s = 0.469$; $P = 0.125$). The correlation between percentage of nongravid adults and percentage of gravid females was nonsignificant with and without the lag ($r_s = 0.161$; $P = 0.618$ in both cases).

The rainfall pattern during this study was significantly different from the 17-yr average (Paired *t*-test: $t = 2.29$; df = 11; $P < 0.05$). The average monthly precipitation during this study was 47.79 ± 23.48 mm, whereas the 17-yr average was 72.66 ± 21.16 mm. Rainfall is generally lowest in June, July, and August, and in December, January, and February; rainfall is highest in April and May and in October and November (López-Ortiz, 1999; Rodriguez-Ramirez and Lewis, 1991).

The presence of hatchlings in all months and gravid females in all months but January shows that reproduction in *S. nicholsi* occurs through most of the year at Cabo Rojo in southwestern Puerto Rico. However, the peak of gravid females in the summer and the peak of hatchlings in the winter suggest strong seasonality.

The observed pattern indicates that hatchlings are most abundant during those months when heat stress is presumably least. The strongest statistical associa-

tion with a climatic variable was the negative relation between day length and percentage of hatchlings. Day length is a stable, reliable indicator of potential for heat stress. Long days occur during those months when the sun is directly overhead; the radiation is both more intense and present for more hours per day than during months with short days. The abundance of *S. nicholsi* across the Cabo Rojo Refuge is negatively associated with substrate temperature. High-density aggregations occur only where the canopy provides dense shade (López-Ortiz, 1999). Hatchlings with extremely small body size and low thermal inertia may be particularly vulnerable to heat stress and high EWL during the long-day months. Their greater relative abundance during the short-day months combined with appropriate microhabitat selection may minimize the problem of evaporative water loss in small sphaerodactylid documented by MacLean (1985).

Peak abundances of gravid females and hatchlings for *S. nicholsi* at Cabo Rojo are one to two months later than those reported for *S. townsendi* at Cabezas de San Juan in Fajardo, northeastern Puerto Rico (Gaa-Ojeda, 1983). Climate patterns are similar at both sites, but Cabo Rojo tends to be drier in June, July, and August when hatchling numbers were lowest. In her relatively small sample, Gaa-Ojeda found hatchlings and small juveniles of *S. townsendi* only from September to November. Oviductal eggs and enlarged follicles were found only from January to June. She suggested that eggs could be incubated during hot months. The pattern we observed in *S. nicholsi* also allows the eggs, which are considered highly resistant to desiccation (Dunson and Bramham, 1981), to incubate during hot, dry summer months.

Members of this genus have one egg per clutch, and presumably several eggs might be produced by an individual in the same reproductive season (Fitch, 1970). Additional observation is needed to evaluate the apparent six month lag between peak abundance of gravid females and peak abundance of hatchlings in the population. Gaa-Ojeda (1983) reported a near four-month incubation time for eggs of *S. townsendi* laid and hatched in laboratory. If a comparable time is required for hatching in nature, the total time between ovulation and active hatchlings may approach the six-month pattern suggested by our data.

The increase in SVL of the population in the months from January to August is a consequence of the growth of individuals and is not just a decline in relative abundance of hatchlings over those months. The size of nongravid adults increased from January to August. The conversion of hatchlings to nongravid adults would reduce average size for nongravid adults if the larger members of the class were not also growing. The erratic decline in median SVL from August with the abrupt drop to the relatively small median SVL of January, when hatchlings made up nearly 50% of the population and no gravid females were encountered, suggests a pattern in which much of the population is replaced each year. Additional study is needed to determine how closely *S. nicholsi* resembles an annual species.

The seasonality in reproduction that we observed is widespread in tropical and temperate species of reptiles and has been shown in an unrelated species in southwestern Puerto Rico (Rodriguez-Ramirez and Lewis, 1991). It may be a consequence of unfavorable climatic conditions that operate indirectly by affecting food supplies or directly by reducing survival of eggs and hatchlings (Colli et al., 1997). Small arthropods, prey of sphaerodactyls, were continuously abundant at the three study sites (López-Ortiz, 1999), and we do not believe the cold season flush of hatchlings is timed to meet a dramatic increase in suitable prey. Similarly, prey abundance is not considered a limiting factor in reproduction of *S. townsendi* (Gaa-Ojeda, 1983). The hard-shelled eggs of *Sphaerodactylus* have evolved tolerance for hot dry conditions (Dunson and Bramham, 1981). We suggest that the annual cycle of day length constrains the annual cycle of *S. nicholsi* through its negative impact on EWL of hatchlings during the hot, dry days of summer and its potentially positive impact on incubating eggs.

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The Provenance of Type Specimens of Extinct Mascarene Island Giant Tortoises (*Cylindraspis*) Revealed by Ancient Mitochondrial DNA Sequences

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Museum collections often contain old and enigmatic specimens that may represent oceanic island endemics that have become extinct since the arrival of human colonists. Most giant tortoise specimens from islands in the Indian Ocean obtained in the first half of the 19th century or earlier lack unequivocal locality data. Consequently, some island species are known certainly only from often fragmentary subfossil remains (Günther, 1877; Arnold, 1979; Bour, 1980, 1984), which have inevitably lost many features through decay. Morphological evidence for the provenance of old nonfossil tortoise specimens without good locality data is often weak or absent, as they frequently consist of isolated shells lacking sufficient diagnostic characters. Ancient DNA extracted from this material provides a new source of characters to establish its locality and affinities.

Accurately assigning old museum tortoise shells to the island populations from which they originated has benefits beyond antiquarian interest, because such specimens can provide information on shell shape, coloring, size at hatching and growth rates. Museum specimens are frequently types, having had formal names coined for them pre-dating those later assigned to subfossils from particular islands, and coordinating all insular tortoise material allows us to rationalize and simplify nomenclature.

Giant land tortoises from the Mascarene islands (Fig. 1) previously placed in the genera *Testudo* and *Geochelone* , are now usually referred to *Cylindraspis,* an apparent clade (Bour, 1984) confined to these islands that is now extinct. Subfossils (Günther, 1877; Arnold, 1979; Austin and Arnold, 2001) show that no less than five species were present: a large saddle-backed form (i.e., with a high front to the shell) and a smaller round-shelled form on Rodrigues; one large saddle-backed form and one large round-shelled form on Mauritius; and a single, rather variable, large species on Réunion. Differences in shell-shape on two-species islands may be related to different browsing proclivities and the degree of openness of the habitats in which the tortoises lived (Arnold, 1979). No museum specimens definitely collected from living pop-

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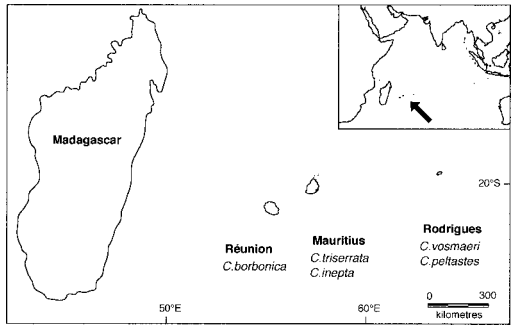


FIG. 1. Map showing the distribution of the five extinct giant tortoise species of the genus *Cylindraspis*.

ulations of *Cylindraspis* are known to exist but a number of complete shells that have been in natural history museums since at least the early 1800s are thought to have been collected in the Mascarenes.

We used mitochondrial DNA (mtDNA) sequences spanning the tRNA-Glu and cytochrome *b* genes to associate some of these museum specimens with subfossil tortoise remains of known provenance. Our results enhance the scientific value of both the old nonfossil and the subfossil material and provide an example of the way museum specimens constantly provide new information in response to advances in technology (Arnold, 1991).

Old Museum Specimens.—(1) Type of *Testudo indica* Schneider, 1783 [Muséum National d'Histoire Naturelle, Paris (MNHN) 7819]. A large high-fronted carapace, 750 mm in straight-line length that has been in the MNHN since at least the early 19th century. There is some circumstantial evidence that the carapace is from the tortoise described and illustrated by Perrault (1676); (see Vaillant, 1893) and originated on Réunion (Froidevaux, 1899; Vaillant, 1900). A Mauritius origin has also been suggested (Boulenger, 1889; Gadow, 1894) and *T. indica* has been regarded as possibly conspecific with Mauritian subfossils now called *Cylindraspis inepta* Günther 1877 (Bour, 1980, 1985).

(2) Type of *Testudo graii* Duméril and Bibron, 1835 (MNHN 9374). A complete, small, rounded shell, 330 mm in straight-line length, which has also been in the MNHM since at least the early 19th century, and was mentioned under the name *Testudo tabulata* var. *africana* (now regarded as a synonym of the South African *Chersine angulata*) by Schweigger (1812). The shell is not fully ossified and is thus likely to be from an immature tortoise with large adult body size; it also shows marked, presumably congenital, abnormalities in the arrangement of dorsal scutes. *Testudo graii* has also been doubtfully allocated to one of the subfossil Mauritian giant tortoise species, *Testudo triserrata* Günther 1877 (Boulenger, 1889; Bour, 1984).

(3) Type of *Testudo vosmaeri* Suckow, 1798 [Nationaal Natuurhistorisch Museum, Leiden (RMNH) 6001]. An intact high-fronted shell 850 mm in straight-line length has been in the natural history museum in Leiden since the 18th century. It has usually been believed to be conspecific with the larger of the two Rodrigues species now known as *Cylindraspis vosmaeri* (Günther, 1877; Arnold, 1979) but a resemblance has also been noted to the subfossil *Cylindraspis borbonica* Bour 1978 from Réunion (Bour, 1980).

(4) Type of *Testudo peltastes* Duméril and Bibron, 1835 (MNHN 7831). Described from another complete shell in the MNHN, this specimen is dome-shaped and 390 mm in straight-line length, and dates from at least the early 19th century. Following Vaillant (1893), *T. peltastes* has generally been regarded as the valid name for the smaller Rodrigues subfossil species.

Subfossil Material.—(1) Réunion. Humeri from three individuals of *Cylindraspis borbonica* Bour, 1978, from Marais de l'Hermitage, Saint-Gilles [Natural History Museum, London (BMNH) 2000.47–49], collected by R. Bour in 1995. Radiocarbon dates from other associated bones vary from 1755 ± 40 years before present (B.P.) to 365 ± 35 yr B.P. (Mourer-Chauviré et al., 1999). *Cylindraspis borbonica* became extinct about 1700 on the western coast, around 1770 on the eastern coast, and about 1830 in the remote higher parts of Réunion (Bour, 1981).

(2) Rodrigues. Shell fragments of one individual of the larger species, *Cylindraspis vosmaeri* (BMNH 2000.50), and of three individuals of the smaller one, *Cylindraspis peltastes* (BMNH 2000.51–53), from caves on the Plaine Corail, Rodrigues, all collected by E. N. Arnold, J. J. Austin, and C. G. Jones in 1997. Both species were extinct by about 1795 (North-Coombes, 1986).

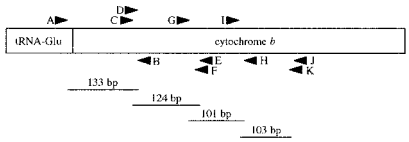
(3) Mauritius. A femur assignable to *Cylindraspis inepta* collected in the mid-1800s from the marsh of Mare aux Songes, southeast Mauritius (BMNH R4021) and a vertebra not assignable to species collected by C. G. Jones from the small cave on Ile aux Aigrettes, southeast Mauritius (BMNH 2000.55) in 1995. Ten other tortoise bones from Mare aux Songes were processed but failed to yield DNA. Radiocarbon dates for other bones from the Mare aux Songes deposit vary from 1490 ± 230 B.P. to 1580 ± 250 B.P. (Burleigh and Arnold, 1986). Tortoises disappeared from the main island of Mauritius and its inner islets about 1680 and from its outer islets about 1735 (Stoddart and Peake, 1979; Bour, 1984).

Other Material.—MtDNA sequences [386–428 base pairs (bp)] from 14 other tortoise species within the Testudinidae were compared with those from old museum specimens and from Mascarene subfossils. Sequences were obtained by us for *Aldabrachelys grandidieri* and *A. gigantea*, whereas sequences for the remainder were taken from the literature (Momont, 1998; Caccone et al., 1999). Further details of material are given in Table 1.

Laboratory Protocols.—Molecular work was carried out following strict procedures to minimize contamination (Austin et al., 1997a,b), in a laboratory dedicated to the study of ancient DNA that was physically separated from all other molecular biology facilities. Tissue samples collected from shells in museums consisted of small pieces of bone or dried soft tissue found adhering to the inner surface of the shell. Bone samples were collected from subfossil shell and limb bones using a rotary cutting wheel or a drill-bit attached to a hand drill (Dremel) after the outer surface of the bone was removed with a grinding wheel to eliminate surface contamination. Bone pieces were ground to powder in a pre-sterilized coffee-mill. Initial sample sizes varied from $<$ 10 mg to 100 mg.

TABLE 1. Giant tortoise material used in the present study. Specimen collected on Aldabra by D. Bourn marked "a."

Species	Locality (? indicates uncertainty)	Source
Old museum specimens		
Testudo indica	Réunion/Mauritius ?	MNHN 7819, museum shell
Testudo graii	Mauritius ?	MNHN 9374, museum shell
Testudo vosmaeri	Rodrigues/Reunion ?	RMNH 6001, museum shell
Testudo peltastes	Rodrigues ?	MNHN 7831, museum shell
Subfossil material		
Cylindraspis borbonica	Réunion	BMNH 2000.47, subfossil humerus
	Réunion	BMNH 2000.48, subfossil humerus
	Réunion	BMNH 2000.49, subfossil humerus
Cylindraspis inepta	Mauritius	BMNH R4021, femur
Cylindraspis sp.	Mauritius	BMNH 2000.55, subfossil vertebra
Cylindraspis vosmaeri	Rodrigues	BMNH 2000.50, subfossil plastron
Cylindraspis peltastes	Rodrigues	BMNH 2000.51, subfossil shell
	Rodrigues	BMNH 2000.53, subfossil plastron
	Rodrigues	BMNH 2000.52, subfossil plastron
Aldabrachelys grandidieri	Madagascar	BMNH 92.6.5.1, subfossil limb-bone
Extant species		
Aldabrachelys gigantea	Aldabra/Seychelles	skull bone[a]
Astrochelys yniphora	Madagascar	Caccone et al., 1999
Astrochelys radiata	Madagascar	Caccone et al., 1999
Geochelone pardalis	Africa	Caccone et al., 1999
Geochelone sulcata	Africa	Momont, 1998
Geochelone elegans	India	Momont, 1998
Chelonoidis nigra	Galápagos	Caccone et al., 1999
Chelonoidis denticulata	South America	Momont, 1998
Chelonoidis carbonaria	South America	Momont, 1998
Indotestudo forsteni	India	Momont, 1998
Manouria emys	Southeast Asia	Momont, 1998
Gopherus polyphemus	North America	Caccone et al., 1999
Pyxis arachnoides	Madagascar	Caccone et al., 1999
Acinixys planicauda	Madagascar	Caccone et al., 1999



Identifier	Sequence	Position[a]	Source
A	TGA CTT GAA RAA CCA YCG TTG	14724	Palumbi, 1996
B	TGT AGG ATT AAG CAG ATG CCT AGT	14854	this study
C	ATC CAA CAT CTC AGC ATG ATG AAA	14841	Kocher et al., 1989
D	CAT CTC AGC ATG ATG AAA CTT CGG A	14848	this study
E	TCG GAT AAG TCA CCC GTA CTG	14966	this study
F	AAG TCA TCC GTA TTG TAC GTC TCG	14957	this study
G	ACT AGC ATT CTC ATC AGT AG	14946	Shaffer et al., 1997
H	GGT AAG AGC CGT ART AAA GTC	15048	this study
I	TGC ATT TAC CTC CAY ATY GGC CG	15045	modified from Shaffer et al., 1997
J	CCC TCA GAA TGA TAT TTG TCC TCA	15149	Palumbi, 1996
K	TCA GAA TGA TAT TTG TCC CCA TGG T	15145	this study

FIG. 2. Location, orientation and sequence of PCR primers used to amplify four overlapping fragments spanning the mitochondrial tRNA-Glu and cytochrome *b* genes in tortoises. Primers B, D-I, and K were designed to be tortoise specific. PCR target sizes exclude the primer sequences and are the largest size amplified for that particular fragment. [a]Position of the 3′ nucleotide of each primer in the complete human mitochondrial genome sequence (Anderson et al., 1981).

Dried soft tissue (~25 mm^2) was rehydrated in 1 ml of 10 mM Tris (pH 8.0) on a rotary mixer at room temperature for 1–2 h, and then macerated with a sterile scalpel blade. Bone powder (0.1–1.0 g) was decalcified in 10 volumes of 0.5 M EDTA (pH 8.0) on a rotary mixer at room temperature for 24–72 h. The EDTA was changed 1–2 times for dark-colored samples (the dark color presumably resulting from soil-derived organics, such as humic acid; Goodyear et al., 1994) until no further color leached from the material. The decalcified bone powder was washed once with 1 ml of 10 mM Tris (pH 8.0) to remove excess EDTA.

DNA was extracted, purified and concentrated either by proteinase K digestion, phenol/chloroform extraction and centrifugal dialysis (Cooper, 1994) or using a Qiamp Tissue Extraction kit (Qiagen) according to the manufacturer's instructions. DNA extracts were stored in 50–200 μl of 10 mM Tris (pH 8.0) at −20°C. An extraction control, without the addition of any tissue or bone powder, was included alongside all DNA extraction attempts.

Four, short (100–130 bp), overlapping fragments of mtDNA, spanning the tRNA-Glu and cytochrome *b* genes, were amplified using two rounds of PCR (Fig.

2). Each fragment was amplified using at least one PCR primer designed to exclude human DNA (the most likely, and most common, form of contamination). Larger amplifications, incorporating two or more of the subfragments were possible with some specimens. Primary amplifications were done using wax-mediated or heat-activated hotstart in 25 μl reaction volumes containing 2.0 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5 μM of each primer, 1 × PCR Buffer (Promega Buffer B or AmpliTaq Gold Buffer), 0.2 mg/ml of BSA, 0.5 units of Taq (Promega) or AmpliTaq Gold (Perkin Elmer) DNA polymerase and 2–5 μl of DNA extract. Cycling parameters were 2 min at 94°C (wax hotstart) or 10 min at 95°C (AmpliTaq Gold hotstart), followed by 40 cycles of 30 sec at 94°C, 40 sec at 50°C or 55°C, 45 sec at 72°C, with a final 5 min extension at 72°C. One microlitre of the primary amplification was added to a 25 μl secondary PCR reaction with identical components and cycling conditions, but excluding BSA and hotstart. PCR primers in the secondary amplification were either identical or internal to primers used in the primary amplification. A PCR negative control, without the addition of any DNA, was included alongside all PCR attempts. Amplification products of the expected size were gel purified using 2% low-melt TAE agarose gels and either a Qiaex II Gel Extraction kit (Qiagen) or a silica-based method (Boyle and Lew, 1995). Both strands of each PCR product were directly sequenced using an ABI Big Dye Terminator Cycle Sequencing kit (Perkin Elmer) with 20–40 ng of DNA, 1.6 pmol of PCR primer, and 1 μl of sequencing mix in a total volume of 10 μl. Sequencing reactions were analyzed on an ABI 373 or 377 DNA Sequencer (Perkin Elmer), and contig assembly/data editing done using Sequencher 3.1.1 (Gene Codes Corporation). Replicate and independent DNA extractions and PCR amplifications were conducted on all specimens to authenticate sequences obtained from initial extraction attempts.

Sequence Analyses.—Sequences were aligned by eye with a single, 2bp indel at the tRNA-Glu/cytochrome *b* junction required in some species. Phylogenetic relationships between all museum and subfossil specimens and all available Indian Ocean island and African species were estimated using the neighbor-joining algorithm (Saitou and Nei, 1987) with Tamura-Nei corrected distances (Tamura and Nei, 1993), implemented in PAUP*4.0b (D. L. Swofford, 2000). Support for internal branches in the tree was estimated by bootstrapping using 1000 replicates (Felsenstein 1985). Sequences have been deposited in GenBank (Accession Numbers: AF371240–241, AF371243–247, AF371250–251. AF371253–257, AF371260).

A total of 428 bp of mtDNA sequence was obtained, including 17 bp of the tRNA-Glu gene, 6bp of intergenic sequence and 405 bp of the cytochrome *b* gene. Sequence identity and the molecular phylogeny (Fig. 3) unambiguously identify the origin of all of the enigmatic museum specimens. Sequences from the types of *T. indica* and *T. graii* are identical and are most similar to sequences from the three Réunion subfossil bones, differing by only one or four nucleotide substitutions (0.2–0.9% sequence difference). This indicates that Réunion is almost certainly the origin of these two shells as Froidevaux (1899) and Vaillant (1900) presumed. Sequence from the type of *Testudo vosmaeri* is identical to that from the bone representing the larger Rodrigues species (usually known as *Cylindraspis vosmaeri)*, whereas the sequence from the type of *T. peltastes* is identical to sequences from the three bones of the smaller species on Rodrigues (usually known as *C. peltastes*). This confirms that both types come from Rodrigues. Their sequences differ by nine nucleotide substitutions (2.1% sequence divergence), all of which are transitions, a degree of difference that corroborates their separate species status. Sequences from the Mauritian *C. inepta* and the unidentified vertebra differ from each other by only two nucleotide substitutions. Sequence difference between specimens from different islands are all greater than within-species comparisons: the two Mauritian sequences differ from *T. indica, T. graii*, and Réunion subfossils by 6–9 substitutions (1.4–2.1%), and from *T. vosmaeri, T. peltastes*, and subfossils from Rodrigues by 25–26 nucleotide substitutions (5.8–6.1%). The five Réunion sequences differ from the six Rodriguan sequences by 21–24 substitutions (4.9–5.6%). All of the Mascarene island tortoise sequences differ from other tortoise sequences examined here by 27–52 nucleotide substitutions (7.0–12.8%). Molecular phylogeny further corroborates the Mascarene island origin for all of the museum specimens. All of the Mascarene tortoises form a relatively well-supported monophyletic clade

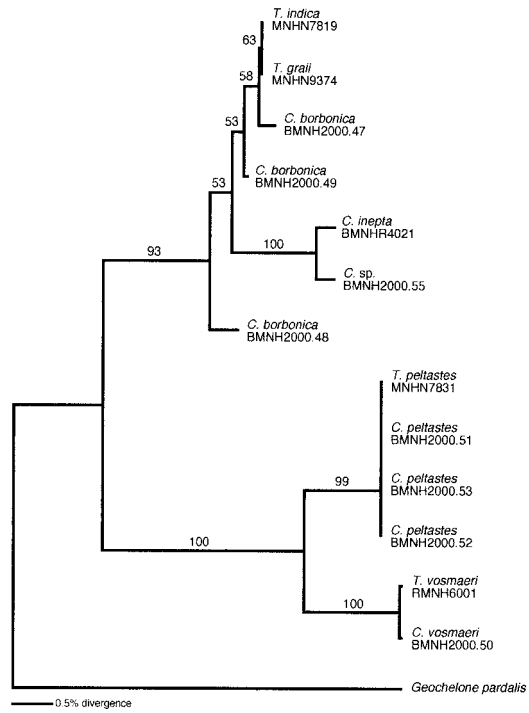


FIG. 3. Neighbor-joining tree for 428bp of mtDNA cyt *b* sequence from the Mascarene and enigmatic tortoise specimens investigated here. Branch lengths are proportional to Tamura-Nei estimates of genetic distance. Bootstrap proportions greater than 50% are shown above branches. The tree is rooted using the African *Geochelone pardalis.*

separate from other Indian Ocean island and African tortoises (Austin and Arnold, 2001).

Biological inferences.—The types of *T. indica* and *T. graii* are essentially uniform in coloring, being rufous to greyish- or blackish-brown. In this uniformity they match shells from Rodrigues and other previously known island giant tortoise populations, but differ from most continental species which often have contrasting shell patterns.

The shells of *T. indica* and *T. graii* are different in shape, indicating that at least some young animals in the Réunion population were rounded, whereas adults could be high-fronted. Numerous fragmentary subfossil shells in MNHN show that some other mature shells are distinctly more domed than the type of *T. indica*, so there may well have been dimorphism that was probably sexual. Such intraspecific variation in shell shape also occurs in *C. vosmaeri* of Rodrigues and in Galápagos giant tortoises.

Three of the shells investigated have horny scutes on their exterior that are smooth as in many mature giant tortoises from populations outside the Mascarenes, but the apparently immature type of *T. graii* shows well-defined growth rings on each scute. Different scutes exhibit approximately 30 rings. In the Mascarenes, where there are marked wet and dry seasons during the year, introduced species of tortoise (for instance *Asterochelys radiata)* usually add one growth ring annually (C. G. Jones, pers. com.), suggesting that the type of *T. graii* was about 30 years old at death. Its scutes also show a central roughened area, the areola, which represents the size of the scute when the tortoise emerged from the egg. For instance, on the second pair of costal scutes, the areola is 13.5 mm $\times$ 20 mm, whereas the scutes themselves are 62 $\times$ 94 mm. The shell of the hatchling was consequently about 21–22% of the 330 mm length of the animal when it died, about 70 mm. This is in agreement with the size of subfossil eggs from Réunion collected at Boucan Canot, Saint-Paul (MNHN 1999.9757), which are about 74–80 mm $\times$ 50–52 mm. Growth rate in the type of *T. graii* was consequently modest, the shell length increasing by an average of about 9 mm per annum. This and other data presented here confirm historical accounts (see compilations in Bour, 1981; North-Coombes, 1986; Cheke, 1987).

Nomenclature.—The earliest name available for the Réunion giant tortoise population is *T. indica* while *T. graii* is a junior synonym of this form, as is *Cylindraspis borbonica.* The latter name has been used consistently in the past 20 yr but not frequently, so it is reasonable to replace it with the senior synonym in the combination *C. indica*. Molecular data also show that the valid names for the large and small Rodrigues species are those currently in use, *C. vosmaeri* and *C. peltastes.* Because neither the names *T. indica* or T. *graii* apply to Mauritian species, the names already employed for these, *C. triserrata* and *C. inepta,* should continue to be used, at least until various other relevant names have been fully assessed, including *T. rotonda* Latreille, 1801, *T. neraudii* Gray, 1831, *T. leptocnemis* Günther, 1875, and *T. boutonii* Günther, 1875.

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Taxonomic Observations on Extant Species and Subspecies of Slider Turtles, Genus *Trachemys*

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Slider turtles, *Trachemys*, have the widest distribution of any turtle genus in the New World. They range from Michigan to Argentina (Fig. 1), and approximately 26 extant forms are known. Most of them are recognized as subspecies of the megaspecies *Trachemys scripta* (Ernst, 1990). The taxonomy of sliders has been a source of confusion for much of the past century. However, within the last 15 years, advancements have been made toward stabilizing generic assignment (Seidel and Smith, 1986) and identification of species/subspecies (α taxonomy) in Mesoamerica (Legler, 1990), the West Indies (Seidel, 1988), and South America (Pritchard and Trebbau, 1984; Vanzolini, 1995). Nevertheless, questions and controversy remain regarding the species/subspecies status of many of the forms. Resolution has been difficult because all of the recognized taxa are either allopatric or parapatric, with only rare or questionable cases of sympatry (Bogert, 1961; Degenhardt and Christiansen, 1974; Ward, 1980; Seidel et al., 1999). A simple solution would be to recognize all of the allopatric subspecies as species (e.g., Collins, 1990; Frost and Hillis, 1990);

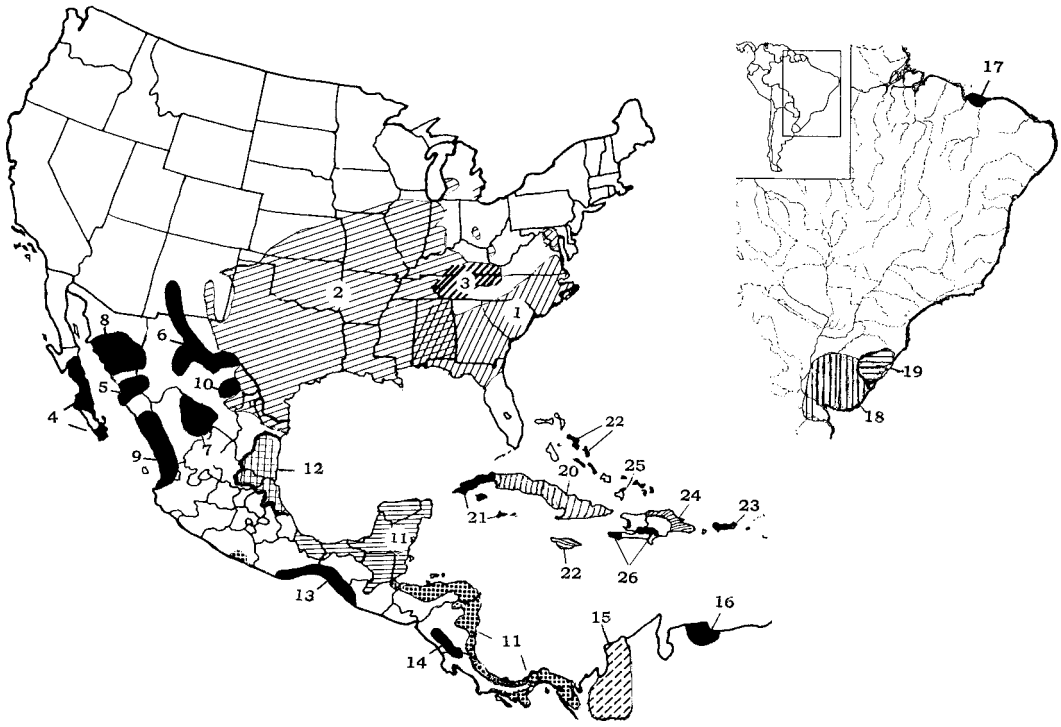

FIG. 1.Distribution of extant species and subspecies of Trachemys. Maps and ranges are modified from Seidel (1988, 1989), Ernst (1990), Legler (1990) and Iverson (1992). Numbers represent taxa listed in Table 2.

however, in my view removing a taxonomic level reduces the hierarchical and biogeographic information content of species nomenclature.

Most authors have recognized the West Indian sliders as several species, distinct from *T. scripta* (sensu Seidel, 1988). However, continental forms, ranging from the United States through Central America to South America have been considered by some a single polytypic species, *Pseudemys (Trachemys) scripta* (Smith and Smith, 1979; Legler et al., 1980; Legler, 1990), whereas others have elevated various subspecies to species rank: *T. dorbigni* (see Seidel, 1989), *T. gaigeae* (see Ernst, 1992), *Pseudemys grayi* (Williams, 1956; Wermuth and Mertens, 1961), *Pseudemys, Chrysemys*, or *Trachemys ornata* (Wermuth and Mertens, 1961, 1977; Weaver and Rose, 1967; Holman, 1977; Fritz, 1990), *Pseudemys* or *Chrysemys callirostris* (Mertens and Wermuth, 1955; Holman, 1977), and the more recently described *T. adiutrix* (Vanzolini, 1995). Partial justification has come from recognizing different courtship behavior (Davis and Jackson, 1973; Fritz, 1990; Ernst et al., 1994; Seidel and Fritz, 1997) and reproductive isolation in captivity (Alvarez del Toro, 1972). However, most species interpretations have not been tested, nor universally adopted. Furthermore, no comprehensive phylogenetic (cladistic) analysis of the continental forms of *Trachemys* has been attempted.

General consensus suggests that slider turtles (*Trachemys*) can be divided into three major groups: United States populations (north and east of the Rio Grande), West Indian populations, and Meso-South American populations (Legler, 1990). Recent morphological, courtship, and genetic data (Stuart and Miyashiro, 1998; Seidel et al., 1999; M. R. J. Forstner, pers. comm.) have demonstrated that *T. s. elegans* and *gaigeae* (both inhabitants of the Rio Grande system in Texas) belong to different species. Further evidence indicates that some of the tropical forms are strongly divergent, whereas others are closely related. These observations are not reflected by current nomenclature, and a taxonomic revision of *T. scripta* is long overdue. The objectives of the present paper are to examine morphological relationships using phylogenetic analysis; evaluate recent results based on phenetic morphology, courtship behavior, and DNA analysis; and propose a species-level taxonomy for the genus. The intent is not to present a definitive phylogeny for *Trachemys* but rather to resolve the nomenclatural inconsistencies and provide a more informative and perhaps more stable assignment of species. A phylogenetic species concept is generally followed but several subspecies are retained for terminal taxa with questionable evolutionary trajectories and absence of clearly defined apomorphies. In these cases explicit intraspecific relationships are not implied.

For phylogenetic analysis, all 26 currently recognized taxa throughout the range of *Trachemys* (Fig. 1) were examined, including more than 1200 specimens (see Appendix 1). Fifty-two characters, based on markings, shell measurements, soft anatomy and osteological features, were initially examined. Smith and Smith (1979), Seidel (1988), Legler (1990), and Seidel et al. (1999) have demonstrated that these kinds of characteristics are useful in distinguishing forms of

TABLE 1. Matrix for cladistic analysis of *Trachemys.* Characters A–W are identified below and states are indicated present (1–3) or absent (0). The primitive (plesiomorphic) conditions are considered to be the states present in *Pseudemys* and/or *Graptemys* (the outgroup) and the other character states are considered derived (apomorphic). A, plastron length/carapace length > 0.89 (1). B, cervical scute underlap (ventral length)/carapace length (1 = <0.035, 2 = 0.037–0.050, 3 = >0.055). C, vertebral scute I anterior width/carapace length (0 = <0.150, 1 = 0.154–0.167, 2 = >0.175). D, male foreclaw length (third ungual manus)/carapace length (0 = 0.036–0.060, 1 = 0.077–0.086, 2 = >0.090). E, male snout length (from orbit)/maximum head width > 0.290 (1). F, maximum cranium depth/condylobasal length > 0.315 (1). G, maximum female carapace length > 350 mm (1). H, squamosal bone tapered posterodorsally (0), blunt posterodorsally (2), or intermediate (1) (Seidel, 1988). I, pygal bone extended beyond marginal-vertebral seam (Hay, 1908) (1). J, mandibular tomium serrate (1). K, choanal papilla present (Parsons, 1968) (1). L, old males with solid black (melanistic) posterior carapace (Seidel, 1988) (1). M, ocellate yellow-orange lines with dark borders (1) or reticulations (2) on carapace. N, dark line symmetrical (dendritic) plastron figure (0), partially disconnected dark spots or ocelli (1), isolated spots or ocelli (2). O, supratemporal (postorbital) stripe yellow (0), orange (1), or red (2). P, supratemporal stripe contacts orbit of eye (1). Q, yellow ''Y'' figure on gular surface (1). R, ventral surface of mandible rounded (1) not flat (0). S, upper (alveolar) surface of mandible broad (1). T, cutting surface of upper jaw not cusped but medially forms an angle or shallow notch (1). U, zygomatic arch relatively narrow (1). V, narial opening of cranium relatively narrow (1). W, entoplastron not elongate (0), at least as broad as it is long (Jackson, 1988). Characters R–V (Seidel and Smith, 1986).

Characters	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
Outgroup	1	2	1	2	0	1	1	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	1
elegans	1	3	1	1	0	1	0	2	1	0	0	1	0	2	2	1	1	1	0	1	1	1	0
scripta	1	3	0	2	0	1	0	2	1	0	0	1	0	2	0	1	1	1	0	1	1	1	0
gaigeae	1	1	2	0	1	1	0	0	1	0	0	0	1	0	1	0	1	1	0	1	1	1	0
taylori	0	1	2	0	0	1	0	2	0	1	0	0	1	0	2	1	1	1	0	1	1	1	0
hartwegi	1	1	2	0	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	1	1	0
yaquia	1	2	2	0	0	1	0	0	1	1	0	0	1	0	1	1	1	1	0	1	1	1	0
nebulosa	0	1	1	0	1	1	1	0	1	0	1	0	2	1	1	0	0	1	0	1	1	1	0
hiltoni	0	1	1	0	1	1	1	0	0	0	1	0	2	1	1	0	0	1	0	1	1	1	0
ornata	0	1	2	0	0	1	1	0	1	1	0	0	1	0	1	1	1	1	0	1	1	1	0
cataspila	0	1	2	0	1	1	0	1	0	1	0	0	1	0	0	1	1	1	0	1	1	1	0
venusta	1	1	2	0	0	0	1	1	0	1	0	0	1	0	0	1	1	1	0	1	1	1	0
grayi	1	2	2	0	1	0	1	2	0	1	0	0	1	0	0	1	1	1	0	1	1	1	0
emolli	0	1	2	0	0	0	1	1	1	0	0	0	1	0	1	0	1	1	0	1	1	1	0
callirostris	1	2	1	0	0	1	0	2	0	1	0	0	1	0	2	0	0	1	0	1	1	1	0
chichiriviche	0	2	1	0	0	1	1	2	0	1	0	0	1	0	2	0	0	1	0	1	1	1	0
dorbigni	1	2	2	0	0	1	0	2	0	0	0	0	1	0	1	1	1	1	0	1	1	1	0
decussata	0	2	0	1	0	0	1	0	0	0	0	0	1	0	1	0	1	1	0	1	1	1	0
terrapen	0	2	2	1	0	1	0	2	0	0	0	0	1	0	1	0	1	1	0	1	1	1	0
decorata	0	2	2	1	1	1	1	2	0	0	0	0	1	2	0	0	1	1	0	1	1	1	0
stejnegeri	0	2	1	2	1	1	0	2	0	0	0	1	1	0	2	0	1	1	0	1	1	1	0
vicina	0	2	1	2	1	1	0	2	0	0	0	0	1	0	2	0	1	1	0	1	1	1	0
malonei	0	2	0	2	1	1	0	2	0	0	0	0	1	0	2	0	1	1	0	1	1	1	0
angusta	0	2	0	1	0	0	1	0	0	0	0	0	1	0	1	0	1	1	0	1	1	1	0
troostii	1	3	1	2	0	1	0	2	1	0	0	1	1	2	0	1	1	1	0	1	1	1	0
brasiliensis	1	2	2	0	0	1	0	2	0	0	0	0	0	0	1	1	1	1	0	1	1	1	0
adiutrix	0	2	2	0	0	1	0	2	1	0	0	0	1	0	1	1	1	1	0	1	1	1	0

Trachemys. However, identifying characters that are phylogenetically informative at low taxonomic levels (species/subspecies) is difficult, especially regarding polarization (Arnold, 1981). Because 29 of the 52 characters were not informative due to extensive variation within taxa or excessive variability in the outgroup (questionable polarization), phylogenetic evaluation was based on 23 characters (A–W, Table 1). Cladistic relationships were defined by phylogenetic analysis using parsimony (PAUP vers. 4.0b4a, D. L. Swofford, Sinauer Associates, Sunderland, MA, 1998; and WinClada vers. 0.9.99M244). The parameters included a heuristic search for multiple, equally parsimonious trees via branch-swapping. The maximum number of trees was not designated and all characters received equal weight. Polarities of character states (Table 1) were based on outgroup comparisons and trees rooted with *Graptemys* and *Pseudemys* (evidence that these two genera are the closest outgroups and that *Trachemys* is monophyletic appears in Seidel and Smith, 1986).

Because the number of taxa included was large (26) relative to the number of useful characters (23), it was not surprising that the PAUP algorithm found 86 equally parsimonious trees. Nevertheless, a 50% majority-rule consensus tree (Fig. 2) illustrates hierarchi-

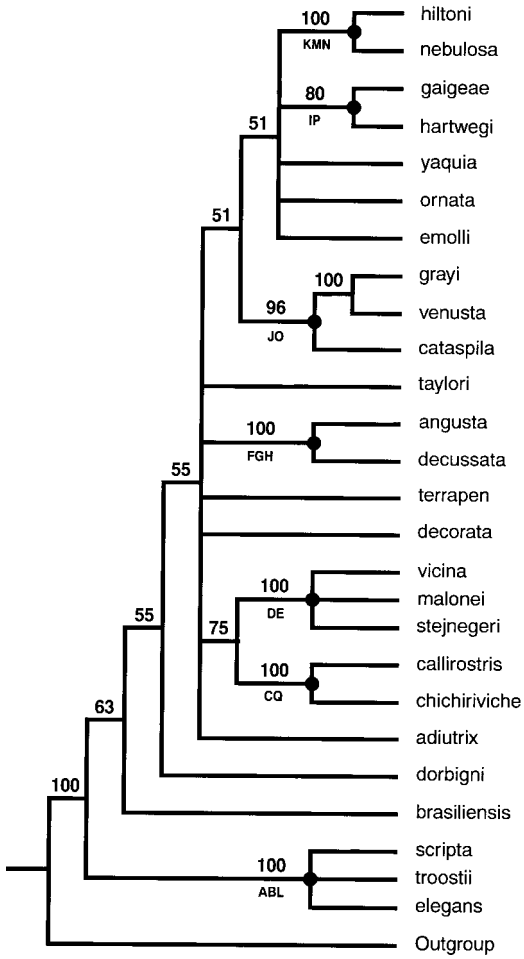

FIG. 2. Fifty percent majority-rule consensus tree (derived from WinClada) rooted with an outgroup of *Pseudemys* and *Graptemys*. The length is 81 and the rescaled consistency index is 0.36. Numbers indicate the frequency (percentage) each branch occurred among all equally parsimonious trees. Solid circles represent nodes for terminal clades with strong support (80–100%), indicative of conspecific taxa (Table 2). Letters represent characters (identified in Table 1), which provide branch support for terminal clades.

cal relationships which are generally congruent with the biogeography of *Trachemys*. Although the tree is polytomous, several major clades are apparent: central and eastern United States forms (*elegans, scripta, troostii*), Northern Mexico isolates (*yaquia, ornata, gaigeae, hartwegi, nebulosa, hiltoni*, dis *emolli*), and a Mesoamerican coastal series (*cataspila, venusta, grayi*). The West Indian *Trachemys* (*terrapen, decorata, decussata, angusta, stejnegeri, vicina, malonei*) appear para- or polyphyletic. Terminal clades which appear in 80–100% of the trees found, (Fig. 2) suggest very close common ancestry, and are here interpreted as polytypic species: *T. scripta* (ssp. *elegans, scripta*, and *troostii*); *T. decussata* (ssp. *decussata* and *angusta*); *T. stejnegeri* (ssp. *stejnegeri, vicina*, and *malonei*); *T. callirostris* (ssp. *callirostris* and *chichiriviche*); *T. venusta* (ssp. *cataspila, venusta*, and *grayi*); *T. gaigeae* (ssp. *gaigeae* and *hartwegi*); and *T. nebulosa* (ssp. *nebulosa* and *hiltoni*). Characters which support these lineages (branches) are identified in Figure 2.

Similar to present results, Legler's (1990) phenetic analysis found morphological similarities among isolated populations in northern Mexico. The inclusion of *emolli* (an isolate from Nicaragua) within this group (Fig. 2) is probably the result of shared homoplasous character states (e.g., B, I or P, Table 1). The divergent position of *taylori* is also consistent with Legler's analysis, as is the clade formed of coastal Mesoamerican sliders (*cataspila, venusta, grayi*). For South American sliders, the sister group of *callirostris* and *chichiriviche* was not surprising considering their similarity and geographic proximity in northern Venezuela (Pritchard and Trebbau, 1984). However, the absence of a *dorbigni, brasiliensis* group was unexpected. The ''outgroup'' positions of *dorbigni* and *brasiliensis* appear to be the result of shared primitive character states and treating them as conspecifics could invoke paraphyly. Nevertheless, these two forms are very similar, broadly disjunct from other sliders (Fig. 1), and may intergrade where their ranges contact in southern Brazil (Seidel, 1989). For these reasons *brasiliensis* is presently retained as a subspecies of *T. dorbigni*. The relationship of *adiutrix* (northern Brazil) to other South American sliders warrants further examination.

Starkey (1997) analyzed mitochondrial DNA in a variety of *Trachemys* and found substantial variation among many of the forms, apparently much more than in the related genus *Pseudemys* (S. K. Davis, unpubl. data). Using a neighbor joining phylogram, Starkey's most divergent lineage was a clade of *scripta, elegans*, and *troostii*, which collectively formed the sister group to *gaigeae*. Although *gaigeae* is not conspecific with these sliders, hybridization (introgression) and some degree of genetic convergence with *elegans* may be occurring in the Rio Grande (Seidel et al., 1999; M. R. J. Forstner, pers. comm.). Hybridization may also be occurring between *elegans* in the Rio Grande system (Rio Salado) and *taylori* in Mexico (Legler, 1963, 1990), although *taylori*, like *gaigeae*, has a courtship behavior different from *elegans* (Davis and Jackson, 1973; Fritz, 1990). Starkey's (1997) results indicate that *taylori, yaquia, emolli*, and *dorbigni* are strongly divergent from each other (separate species?) as well as from other Meso- and South American forms. Unfortunately, *nebulosa, hiltoni, hartwegi, ornata, malonei, adiutrix*, and *brasiliensis* were not sampled. In contrast to Seidel (1988) and present results (Fig. 2), Starkey (1997) suggested that the West Indian species of *Trachemys* are a monophyletic lineage. However, the species relationships of Seidel (1988) are generally upheld by his DNA data. It is noteworthy that both types of male sexual dimorphism (snout and foreclaw elongation), which are presumably related to dichotomous courtship patterns (Legler, 1990; Stuart and Miyashiro, 1998), are represented among West Indian *Trachemys* (Table 1). Divergent reproductive behavior, in addition to the presence of variable forms of male melanism (Seidel, 1988), suggests multiple origins for West Indian sliders.

In conclusion, present results, as well as studies over the last 15 years, indicate that many of the taxa

TABLE 2. A proposed taxonomy for species and subspecies of *Trachemys.* Distinguishing characteristics are given for polytypic species which contain new or restricted combinations of subspecies. See Smith and Smith (1979), Pritchard and Trebbau (1984), Seidel (1988, 1989), Legler (1990), Ernst (1990) and Vanzolini (1995) for descriptions and illustrations. The numbers identify geographic ranges in Figure 1.

	Trachemys scripta (Schoepff), 1792:16—isolated spots or ocelli on plastron, old males with solid black posterior carapace, elongated cervical scute underlap, elongated male foreclaws.
1	*T. s. scripta* (Schoepff), 1792:16
2	*T. s. elegans* (Wied), 1839:213
3	*T. s. troostii* (Holbrook), 1836:55
	Trachemys nebulosa (Van Denburgh), 1895:84—cervical scute underlap short, male snout elongate, markings on carapace irregular or reticulate.
4	*T. n. nebulosa* (Van Denbrugh), 1895:84
5	*T. n. hiltoni* (Carr), 1942:1
	Trachemys gaigeae (Hartweg), 1939:1—supratemporal marking orange and not contacting orbit, plastral pattern reduced and narrow, pygal bone elongate.
6	*T. g. gaigeae* (Hartweg), 1939:1
7	*T. g. hartwegi* (Legler), 1990:89
8	*Trachemys yaquia* (Legler and Webb), 1970:158
9	*Trachemys ornata* (Gray), 1831:30
10	*Trachemys taylori* (Legler), 1960:75
	Trachemys venusta (Gray), 1855:24—vertebral scute broad, mandibular tomium serrate, supratemporal stripe yellow and contacting orbit, carapace ocelli complete.
11	*T. v. venusta* (Gray), 1855:24
12	*T. v. cataspila* (Günther), 1885:4
13	*T. v. grayi* (Bocourt), 1868:121
14	*Trachemys emolli* (Legler), 1990:91
	Trachemys callirostris (Gray), 1855:25—supratemporal stripe red and not contacting orbit, ''Y'' figure absent from gular surface.
15	*T. c. callirostris* (Gray), 1855:25
16	*T. c. chichiriviche* (Pritchard and Trebbau), 1984:191
17	*Trachemys adiutrix* Vanzolini, 1995:112
	Trachemys dorbigni (Duméril and Bibron), 1835:272
18	*T. d. dorbigni* (Duméril and Bibron), 1835:272
19	*T. d. brasiliensis* (Freiberg), 1969:301
	Trachemys decussata (Gray), 1831:28
20	*T. d. decussata* (Gray), 1831:28
21	*T. d. angusta* (Barbour and Carr), 1940:402
22	*Trachemys terrapen* (Lacépède), 1788:129
	Trachemys stejnegeri (Schmidt), 1928:147
23	*T. s. stejnegeri* (Schmidt), 1928:147
24	*T. s. vicina* (Barbour and Carr), 1940:408
25	*T. s. malonei* (Barbour and Carr), 1938:76
26	*Trachemys decorata* (Barbour and Carr), 1940:409

(subspecies) of *T. scripta* are broadly allopatric and morphologically distinct. That, combined with evidence of biochemical divergence (Seidel, 1988; Starkey, 1997) and variation in courtship behavior, implicitly supports recognizing most of these taxa as species. Table 2 presents a proposed taxonomy for sliders in the genus *Trachemys*, partitioned into 15 species, eight of which are polytypic. This represents greater species diversity than previous arrangements and could enhance conservation efforts, especially in the Neotropics where protection is often limited to the species level.

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APPENDIX 1

In addition to the 750+ *Trachemys* specimens listed in Seidel (1988) and Seidel et al. (1999), the following specimens were examined. Abbreviations for museums follow Leviton et al. (1985) except for MES (reference collection of author) and PCHP (collection of Peter Pritchard).

Trachemys scripta scripta.—MES 1952. NCSM 7162, 9268–69, 10358, 11873, 13811, 14939, 15115, 16453, 17580, 19175, 20997–98, 21625, 23082, 23879, 24008, 28885. PCHP 303, 306, 331, 1595, 1699, 2695, 4007. UF 11934, 13502, 13506–07, 13572, 13608, 13669, 30108, 65846. USNM 51358–60, 91308 .

Trachemys scripta elegans.—MES 1787, 1878, 1948–51, 1953. MSB 23773, 37594, 42230, 42417, 42561–62. TCWC 51831, 61776. USNM 83173–77, 83178–79, 83184–86. UU 4164, 17523.

Trachemys scripta troostii.—MES 1946–47, 1954. UMMZ 96261–63, 218733–35. USNM 86717–18, 86724, 86758–59, 86761, 86786–87.

Trachemys nebulosa nebulosa.—MES (uncataloged). PCHP 403–04. USNM 12622, 240694. UU 12450–51, 12749, 14797.

Trachemys nebulosa hiltoni.—KU 46678, 47585–88, 63601, 63604–09. UU 3824–37, 3831–32, 3840, 3845.

Trachemys gaigeae gaigeae.—KU 51202–03, 51208, 51212, 51215, 51219, 51315, 91380. MCZ 31974. MSB 22406, 54750. SRSU 5669, 5930. UU 12406.

Trachemys gaigeae hartwegi.—KU 29357. MCZ 4550–51. UF 39634, 62933–34. USNM 105265, 105267, 105269. UU 4701, 12504, 17583.

Trachemys yaquia.—UF 52756–62. UU 6030, 6033, 12487.

Trachemys ornata.—KU 63610–11, 78979. UF 48172, 22382. USNM 66198, 238183. UU 3803–04, 3810, 3813, 11147–49, 11154, 11375, 12920, 12923.

Trachemys taylori.—KU 53785, 53787. MES 107. MSB 30552–57, 30559, 30561–63. UF 48101–02, 48092–93. USNM 30560, 159579, 166365. UU 3854, 3861, 11253–56.

Trachemys venusta venusta.—CM 62091, 91077–80, 91082, 96016–18, 96029, 96037, 96040, 96043, 96051, 105873–74, 112796–812, 117679–80, 117682–83, 117685–87, 117689, 117693–94, 124230. KU 85532, 102542, 171406. MCZ 7868, 16770–76, 19351–53, 31484, 31963–64, 34356, 53150, 55121, 71641–42. MES 129. MSB 30549–51. PCHP 4196. UF 7689, 10299, 13479–80 (4), 24071, 37161, 40813, 43106, 50473–75, 50477–80, 50809, 90020, 99978–79. USNM 51068, 53883, 54085, 54088, 55603, 61246, 103707, 129587, 129609, 134440, 136612, 222426, 292553. UU 6164, 6598, 6602, 6693, 6832–40, 6842–49, 6859, 6881, 6899, 6923, 6940, 6962, 9659, 9670, 9679, 9681, 9701–03, 11334, 11342.

Trachemys venusta cataspila.—PCHP 1251, 1840. TCWC 26495, 26497–98, 53145–47. USNM 30746.

Trachemys venusta grayi.—MCZ 4982–87. PCHP 4008. USNM 46281–82, 109086. UU 11362, 11367, 11370, 11372–74.

Trachemys emolli.—KU 85531, 128705–06. PCHP 3359, 4732, 4737. TCWC 56903–09. UU 6701, 6760, 6762, 13026.

Trachemys callirostris callirostris.—FMNH 74893–94, 194301. KU 94578. MCZ 54715–16, 57241–48. MES 1711, 1783. PCHP 15828–31, 2772. UF 22375, 22383, 43107, 49088, 49171–79. UMMZ 110564–65, 126861 (8).

Trachemys callirostris chichiriviche.—MCZ 172053.

PCHP 1465, 1470, 1472, 1520, 1524–25, 2662, 2736–37, 4799. UF 53333–34.

Trachemys adiutrix.—USNM 329467.

Trachemys dorbigni dorbigni.—CM 57095–99, 62078, 96001–02. MCZ 1890, 174751. PCHP 2064, 3015. USNM 107788.

Trachemys dorbigni basiliensis.—MCZ 33502, 51445. PCHP 2954, 3181.

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On the Nomenclature of the Skink (*Mabuya*) Endemic to the Western Atlantic Archipelago of Fernando de Noronha, Brazil

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The skink genus *Mabuya* currently comprises more than 100 species worldwide (Greer and Broadley, 2000; Greer and Nussbaum, 2000). Approximately 20 species are known for the Neotropics (Mijares-Urrutia and Arends R., 1997; Greer et al., 2000; Rodrigues, 2000). As is the case for many of the Old World species, systematics of the Neotropical forms remain unsatisfactorily resolved. One of the South American taxa, currently referred to as *Mabuya maculata*, is endemic to the archipelago of Fernando de Noronha, in the western Atlantic, approximately 200 km off the northeast Brazilian coast. Specimens from Fernando de Noronha differ from other *Mabuya* species in numerous morphological features, especially strongly keeled dorsal scales, uniform coloration without a trace of striping, and unusually high dorsal, ventral, and midbody scale counts (e.g., Dunn, 1935; Peters and Donoso-Barros, 1970). As a result, they have been linked to the Cape Verde Islands *Mabuya* species, rather than to any of the New World congeners (e.g., Dunn, 1935; Horton, 1973). In spite of the Noronha species' distinctiveness and restricted geographic range, the question of which is the valid name because it has been much discussed since its first description over 160 years ago and, in our opinion, is not yet resolved.

The *Mabuya* from Fernando de Noronha was first described by Gray (1839) as *Tiliqua punctata*. On the same page of the same work, Gray described a second skink, *Tiliqua maculata*, from Demerara, British Guiana (now Guyana). Since then, both names have been alternatively considered synonymous and distinct by various authors (for a thorough review of the literature see Travassos, 1946, 1948).

Boulenger (1887) was the first to allocate Gray's species to the genus *Mabuya* Fitzinger, 1826, and was also the first to consider them synonymous, based on examination of the types at the British Museum of Natural History (BMNH). Acting as first revisor in the sense of the Code of Zoological Nomenclature, Boulenger gave the name *punctata* priority over *maculata*.

Andersson (1900) noted the homonymy between Gray's species and *Lacerta punctata* Linnaeus, 1758, which he considered a synonym of *Mabuya homalocephala* (Wiegmann); he thus replaced the name *punctata* by its junior synonym *maculata*.

Dunn (1935), apparently unaware of Andersson's work, disputed Boulenger's (1887) synonymy of *maculata* with *punctata* and restored the latter name for the Noronha species; he also synonymized *M. maculata* with *M. mabouya mabouya* (Lacépède, 1788). *Mabuya mabouya mabouya*, as defined by Dunn, is now known to represent a composite of several different species (e.g., Hoge, 1946; Reboucas-Spieker, 1974, 1981; Avila-Pires, 1995; Mayer and Lazell, 2000). Dunn apparently made these decisions based on Gray's descriptions and on the examination of four specimens from Fernando de Noronha and six from the Guianan region. He did not examine Gray's types.

Schmidt (1945) agreed with Dunn's synonymy of *M. maculata* with *M. mabouya* but, considering the homonymy noted by Andersson (1900), proposed the new name *atlantica* to replace the preoccupied name *punctata*.

Travassos (1946) disagreed with Dunn (1935) and considered both of Gray's species indistinguishable; unaware of both Andersson's (1900) and Schmidt's (1945) contributions, he retained the name *M. punctata* (he further presented a synonymy and bibliography). Later, Travassos (1948) again reviewed the literature concerning the Fernando de Noronha *Mabuya*, presenting a more complete reference list. He agreed with Andersson (1900) in considering *Lacerta punctata* L. a *Mabuya*, based on pictures of the type and a letter of U. Bergstrom of the Stockholm Museum of Natural History confirming that the type is indistinguishable from *M. homalocephala*. Nevertheless, Travassos (1948) disagreed with Schmidt (1945) in considering Gray's two species distinct from each other and dropped Schmidt's epithet *atlantica* in favor of the older *maculata*, thus reinstating Andersson's (1900) arrangement. Travassos did not examine Gray's types (two of *M. punctata* and two of *M. maculata*), and his decisions were based on data supplied to him by the British Museum staff. Nevertheless, his arrangement has remained in use ever since, although some recent publications have referred to the Noronha species by the name *punctata* (e.g., Brygoo, 1985; Greer et al., 2000; Greer and Broadley, 2000; Greer and Nussbaum, 2000), apparently caused by the authors not being aware of the last nomenclatural changes (Brygoo, 1985; A. E. Greer, pers. comm.).

Two additional names have been included in the synonymy of *M. punctata* (Gray): *Mabuya punctatissima* O'Shaugnessy, 1874, synonymized by Boulenger (1887) and *Trachylepis* (*Xystrolepis*) *punctata* Tschudi, 1845, synonymized by Travassos (1946). *Mabuya punctatissima* was described by O'Shaugnessy (1874) based

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TABLE 1. Morphometric (in millimeter) and meristic data of types of *Mabuya maculata, Mabuya punctata,* and *Mabuya punctatissima* at the BM and of 100 *Mabuya punctata* (sensu Boulenger, 1887) examined by Travassos (1946), given as ranges and mean values (for morphometric data) or modal values (for meristic data) in parentheses.

Species	*Mabuya maculata* BM 1946.8.18.5	*Mabuya maculata* BM 1946.8.18.6	*Mabuya punctata* BM 1946.8.27.47	*Mabuya punctata* BM 1946.8.27.48	*Mabuya punctatissima* BM 1946.8.18.40	*Mabuya punctata* (data from Travassos, 1946)
Locality	Demerara (Guyana)	Demerara (Guyana)	Fernando de Noronha (Brazil)	Fernando de Noronha (Brazil)	Cape	Fernando de Noronha (Brazil)
Snout–vent length	79	86	87	58	107	58–93 (73.6)
Head length	17.0	17.5	21.4	12.3	21.8	12.0–18.9 (14.8)
Head width	9.9	10.4	9.4	6.7	12.8	7.0–14.4 (9)
Head height	8.1	8.4	8.1	6.2	11.4	—[d]
Tail length	129	73[b]	142	110	150	63–170 (11)
Supranasals	in contact	in contact	in contact	in contact	in contact	in contact
Prefrontals	in contact	separated	in contact	in contact	in contact	in contact in 82% of cases
Frontoparietals	2	2	2	2	2	2
Parietals	separated	separated	in contact	in contact	in contact	in contact
Supraoculars	4–4	4–4	4–4	4–4	4–4	3–4 (4)[e]
Supraciliaries	—[a]	5–5	5–5	5–5	5–5	5–5
Keels on dorsals	5	5	3	3	0–3[c]	3
Midbody scales	34	32	42	40	37	34–40 (38)
Dorsals	57	55	67	68	66	58–69 (63–64)
Ventral	64	62	69	71	66	66–78 (70)
Subdigitals IV finger	15	16	19	23	17	—[d]
Subdigitals IV toe	18	18	29	29	23	21–28 (26)

[a] Not counted because of scale damage.
[b] Tail regenerated.
[c] Keels weakly developed.
[d] Not measured by Travassos.
[e] Three supraoculars present in only one specimen.

on museum material from dubious procedence (locality only called ''Cape''). Dunn (1935) said he was ''unable to comment'' on Boulenger's decision [he did not remove *punctatissima* from synonymy with *M. punctata* as insinuated by Travassos (1948)]. Travassos (1946) provisionally considered *M. punctatissima* synonymous with the Noronha form (then known to him as *M. punctata*) pending further revision. No such revision has since been done, and O'Shaugnessy's name currently remains in the synonymy of *M. maculata* (sensu Travassos, 1948).

Travassos (1946) synonymized *Trachylepis* (*Xystrolepis*) *punctata* Tschudi, 1845 (type locality: Peru) based on Tschudi's (1845) description and illustrations. He disagreed with Boulenger (1887) and Dunn (1935) who both considered Tschudi's species as indeterminate. We will not comment further on *T*. (*Xystrolepis*) *punctata* in the present paper, because no type material of this taxon has been examined by us, and it is a junior homonym within *Mabuya*.

Although the nomenclature of Gray's (1839) species have been reviewed extensively by various authors within more than 100 years, it seems that the types themselves have not been examined since Boulenger (1887).

Recently, the senior author examined the types of *maculata* (BM1946.8.18.5–6), *punctata* (BM1946.8.27.47–48) and *punctatissima* (BM1946.8.18.40). The types of both *punctata* and *maculata* are similar in color pattern: all are olive-brown to grayish with small dark spots all over the body except the venter, and there are no traces of striping. The following measurements were taken (to the nearest 0.1 mm with a dial caliper): snout–vent length, head length, from snout to anterior border of ear opening, head width (taken at the insertion of the mandible), head height (taken at center of eyes), and tail length. Scale counts considered were: midbody scale rows (taken at the midpoint between forelimbs and hind limbs), dorsal scales from nuchals to tail base, ventral scales from postmental to vent, subdigital lamellae under fourth finger, subdigital lamellae under fourth toe. Qualitative characters include presence or absence of keels on dorsal scales (and number of keels, if present), and presence or absence of contact between parietals, prefrontals and supranasals.

Results are summarized in Table 1. The number of keels on the dorsal scales (a character considered important in *Mabuya* systematics) suggests that two species may be involved. Also, the separated parietals in *M. maculata* may distinguish it from *M. punctata*, because this character state is apparently invariable in the latter. Both *M. punctata* syntypes have higher numbers of midbody scales, dorsals, ventrals, and subdigital lamellae under finger IV and toe IV than the *M. maculata* types. Counts for the latter fall well within

the variation observed for various other Neotropical congeners (e.g., Dunn, 1935; Avila-Pires, 1995; Mijares-Urrutia and Arends, 1997) but generally fall outside the lower limit of the variation reported by Travassos (1946) for his Noronha sample (see Table 1). Thus, we strongly suspect that *M. maculata* (Gray) is a species distinct from that inhabiting Fernando de Noronha and that, consequently, that name is not available to designate the Noronha population. The identity of *M. maculata* remains problematic. Travassos (1946, 1948) thought it possible that *M. maculata* (sensu Andersson, 1900) could occur both in Fernando de Noronha and in Demerara, Guyana. Nevertheless, no specimens of this highly distinctive *Mabuya* have ever been recorded in mainland northeastern Brazil or along the intervening area between that region and the Guianas. The type locality of *maculata* should be considered dubious: it was reportedly collected in Demerara by Capt. Sabine, but some other specimens of lizards and snakes from ''Demerara'' collected by Capt. Sabine turned out to be restricted to certain Caribbean islands, including St. Kitts and Nevis and St. Lucia (Underwood, 1993). Of the insular *Mabuya* specimens in the BMNH (excluding the Noronha specimens), only those from St. Lucia and Barbados are without a lateral stripe, so it is likely that the type locality of *maculata* is St. Lucia or Barbados (G. Underwood, pers. comm.). The geographic range of *M. maculata* (sensu Andersson, 1900) was finally restricted to Fernando de Noronha by Peters and Donoso-Barros (1970), who cited the species' type locality as ''Surinam (in error)'' (sic). However, they did not comment on how they concluded that the type locality was wrong. A stripeless *Mabuya* with a dark-spotted dorsal pattern (*M. falconensis*) has recently been described from northern Venezuela (Mijares-Urrutia and Arends, 1997). Thus, in case the type-locality of *maculata* is not wrong (as suggested above), it is worth bearing in mind that black-spotted *Mabuya* species with indistinct or barely evident striping do occur in northernmost South America. It is even possible that the *maculata* types may have been collected in Venezuela, since some of Capt. Sabine's reptile specimens from ''Demerara'' in the BMNH collection belong to species known from various parts of South America but not known from Guyana (G. Underwood, pers. comm.). Thus, we believe that there is a possibility that Gray's *maculata* may be identical to *M. falconensis*, but because we have not seen the types (and only known specimens) of the latter, we cannot speculate any further; moreover, the fact that *M. falconensis* has smooth to slightly carinated scales and scale counts slightly lower than those of *maculata* (see Mijares-Urrutia and Arends, 1997) speaks against that possibility.

The number of presacral vertebrae is of systematic significance within the genus *Mabuya*, American *Mabuya* species having 28 to 33 presacral vertebrae, whereas most of the 61 examined Old World *Mabuya* taxa, as well as the Fernando de Noronha species, have only 26 presacral vertebrae (Greer et al., 2000). Unfortunately, because of their poor state of conservation (both type specimens of *maculata* show a very high degree of chalk sclerosis), it was not possible to radiograph the types of *maculata*. The types of both *punctata* and *punctatissima* have 26 presacral vertebrae, which supports a Noronha or Old World origin for both.

The *M. punctatissima* type has scalation values that are intermediate between those of the *maculata* and *punctata* types in all cases. Nevertheless, they are all within the variation found in the Noronha species (Table 1). The type is larger than the largest specimen examined by Travassos (1946; Table 1) and has dorsal scales, which are either smooth or bear weakly developed keels (in contrast to the well developed keels of Gray's specimens). Hence, we suggest that it should be removed from the synonymy with Gray's species. The *M. punctatissima* type may represent a southern African taxon as the type locality suggests, but identifying its true status is beyond the scope of this paper. In any case, if it proves to represent a valid species not referable to any other taxon, a substitute name will have to be created, because *M. punctatissima* O'Shaugnessy, 1874, is a junior homonym of *Euprepes punctatissimus* Smith, 1849 (currently *M. striata punctatissima*).

In summary, we conclude that *M. maculata* (Gray, 1839) refers to a taxon distinct from that represented by specimens from Fernando de Noronha. However, because of the bad state of the types and the apparent uncertainty regarding its type locality, we are unable to determine its specific identity. *Mabuya punctatissima* O'Shaugnessy, 1874, may also be distinct from the Noronha species and is a junior homonym. Because the name *M. punctata* (Gray, 1839), which does represent the Noronha species, is not available resulting from its preoccupation by *M. punctata* (Linnaeus, 1758), the correct name for the *Mabuya* species from Fernando de Noronha should be *M. atlantica* Schmidt, 1945.

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The Link between Mating Season and Male Reproductive Anatomy in the Rattlesnakes *Crotalus viridis oreganus* and *Crotalus viridis helleri*

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In temperate zone pitvipers of North America, the mating season may be temporally dissociated from fertilization. For example, the mating season in *Crotalus viridis viridis* occurs in summer preceding, by as much as seven months, spring ovulation (Duvall et al., 1985). Mating may also be temporally dissociated from spermatogenesis. Several species of pitvipers (*Agkistrodon contortrix*, Mount, 1975; *Crotalus atrox*, Wright and Wright, 1957) mate in spring, before spermatogenesis begins. Thus, within the pitvipers of North America, the mating season appears independent of gametogenesis. Because males can store sperm in the vas deferens for at least one year (Aldridge, 1993) and females can store sperm in the oviduct for at least eight months (Schuett, 1992), the timing of the mating season may be acted on by natural selection to occur at times most favorable to females.

In almost all North American pitvipers in which the reproductive cycle has been described, vitellogensis begins in the summer, and the follicles overwinter at an intermediate size (Aldridge, 1979). After emergence from hibernation, vitellogenesis resumes, followed by ovulation and fertilization in the spring. The mating season in pitvipers appears to be restricted to the period of vitellogenesis (Rahn, 1942; Schuett, 1992). Thus, mating may occur in the summer, during the early stages of vitellogenesis, or in the spring, during the late stage of vitellogenesis, or during both periods (Schuett, 1992).

Within the widespread western rattlesnake (*Crotalus viridis*), the seasonal pattern of vitellogenesis described above (summer–spring) has been reported for *C. v viridis* (Rahn, 1942; Aldridge, 1979), and *Crotalus viridis oreganus* (Diller and Wallace 1984; Macartney and Gregory, 1988). The only apparent exception to this pattern was presented by Fitch (1949) for a population of *C. v oreganus* in Madera County, California. Fitch (1949) implied that vitellogenesis is restricted to the spring in this population. However, a comparison of the data presented by Fitch (1949) with data presented by Rahn (1942) and Aldridge (1979), for *C. v viridis* in Wyoming and New Mexico, respectively, indicates that Fitch (1949) may have had both reproductive and nonreproductive snakes in his sample. Thus, the presence of vitellogenic and nonvitellogenic females in his early samples indicates that vitellogenesis started in the previous summer in reproductive snakes.

Schuett (1992) identified two major patterns of mating and sperm storage in pitvipers. The first, long-term sperm storage (LTSS), is sperm storage in the oviduct from matings that occur in the summer and fall, prior to hibernation. The second, short-term

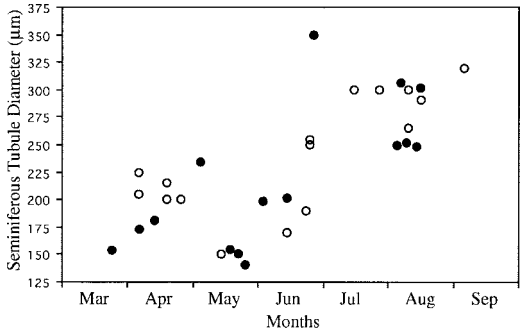

FIG. 1. Diameters of the seminiferous tubule of *Crotalus viridis helleri* (dots) and *Crotalus viridis oreganus* (circles). Each point represents the mean of 12 measurements per snake.

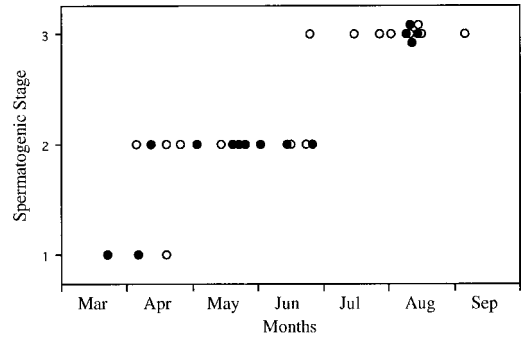

FIG. 2. Spermatogenic stage of *Crotalus viridis helleri* (dots) and *Crotalus viridis oreganus* (circles): 1 = tubules contain primarily spermatogonia, 2 = tubules contain primarily spermatocytes, 3 = tubules contain primarily spermatids and spermatozoa

sperm storage (STSS), is sperm storage in the oviduct from matings that occur in the spring. Both matings seasons, summer and the following spring, occur during the same vitellogenic cycle in the female. The difference between LTSS and STSS is not meaningful with respect to the age of the sperm. Because spermatogenesis occurs in the summer in all species of temperate North American Colubridae and Crotalinae snakes examined (Saint Girons, 1982; Aldridge and Brown, 1995), the age of the sperm, whether snakes mate in summer, spring, or both, is identical. The difference lies in where the sperm are stored. In STSS, sperm are stored in the vas deferens, whereas in LTSS, sperm are stored in the oviduct. Thus, the occurrence of LTSS and STSS is probably a result of natural selection acting on the timing of the mating season and not on the age of sperm used in fertilization.

In his review of the literature on mating seasons in the *C. viridis* complex, Aldridge (1993) noted *C. v. oreganus* and *Crotalus viridis helleri* had a mating season different from populations of *C. v. viridis*. In these subspecies, a spring mating period, as well as a summer mating period, were common. The purpose of this research is to examine the seasonal cycle of the testis and sexual segment of the kidney in the *C. v. oreganus* and *C. v. helleri*, and compare these findings with the published accounts of mating and male-male combat in these subspecies.

Reproductive tracts were obtained from museum specimens (Appendix 1). When available, road killed specimens or specimens of known date of capture and preservation were examined. Thirty-four of the snakes in the sample were from California and one was from Oregon, USA. The right testis and anterior portion of the kidney, with vas deferens attached, were removed, dehydrated in isopropanol, cleared in toluene, embedded in paraffin, sectioned at 7 μm. Tissues were stained in hematoxylin, Biebrich scarlet, orange G, and fast green. Data taken include seminiferous tubule diameter (STD), stage of spermatogenesis, diameter of the sexual segment of the kidney (SSK), and presence of sperm in the vas deferens. Points on Figures 1–4 represent the mean of 12 measurements per snake. To determine differences in the activity of the STD and SSK, the active season was divided into three parts, the early season (March and April), the middle season (May and June) and the late season (July–September). These months were chosen because an analysis by month indicated that the diameters of the STD and SSK did not differ during these time periods.

The comparison of mean snout–vent length (SVL), seminiferous tubule diameter (STD), and diameter of the sexual segment of the kidney (SSK) of *C. v. oreganus* and *C. v. helleri* were compared using the Mann-Whitney *U*-test. All statistics were computed with StatView 4.5 (SAS Institute Inc., Cary, NC). On the combined data of both taxa, seasonal means for SVL, STD, and SSK were analyzed by the Kruskal-Wallis test (with *H*- and *P*-values corrected for ties). Comparisons of means were examined by the Mann-Whitney *U*-test. Results presented are means and standard deviations. For all statistical tests $\alpha = 0.05$.

The overall means of the SVL ($Z = -1.6$, $P = 0.10$), STD ($Z = -1.2$, $P = 0.21$), and SSK ($Z = -1.9$, $P = 0.16$), were not significantly different between of *C. v.*

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FIG. 3. Photomicrographs of cross-sections of the testis (left) and sexual segment of the kidney (right). A and B are sections from a *Crotalus viridis helleri* collected in early May (ASU 30984), seminiferous tubule epithelium (A) has three to four layers of primary spermatocytes, and some developing spermatids, sexual segment of the kidney (B) has many granules and a large lumen; C and D are sections from a *Crotalus viridis oreganus* collected in late June (NHMLA-28029), seminiferous tubule epithelium (C) has two to three layers of primary spermatocytes and > 10 layers of secondary spermatocytes and spermatids, sexual segment of the kidney (B) has many granules and a large lumen; E and F are sections from a *C. v. helleri* collected in early August (MVZ-10537), seminiferous tubule epithelium (E) has many spermatids and developing sperm, sexual segment of the kidney (F) has many granules and a small lumen.

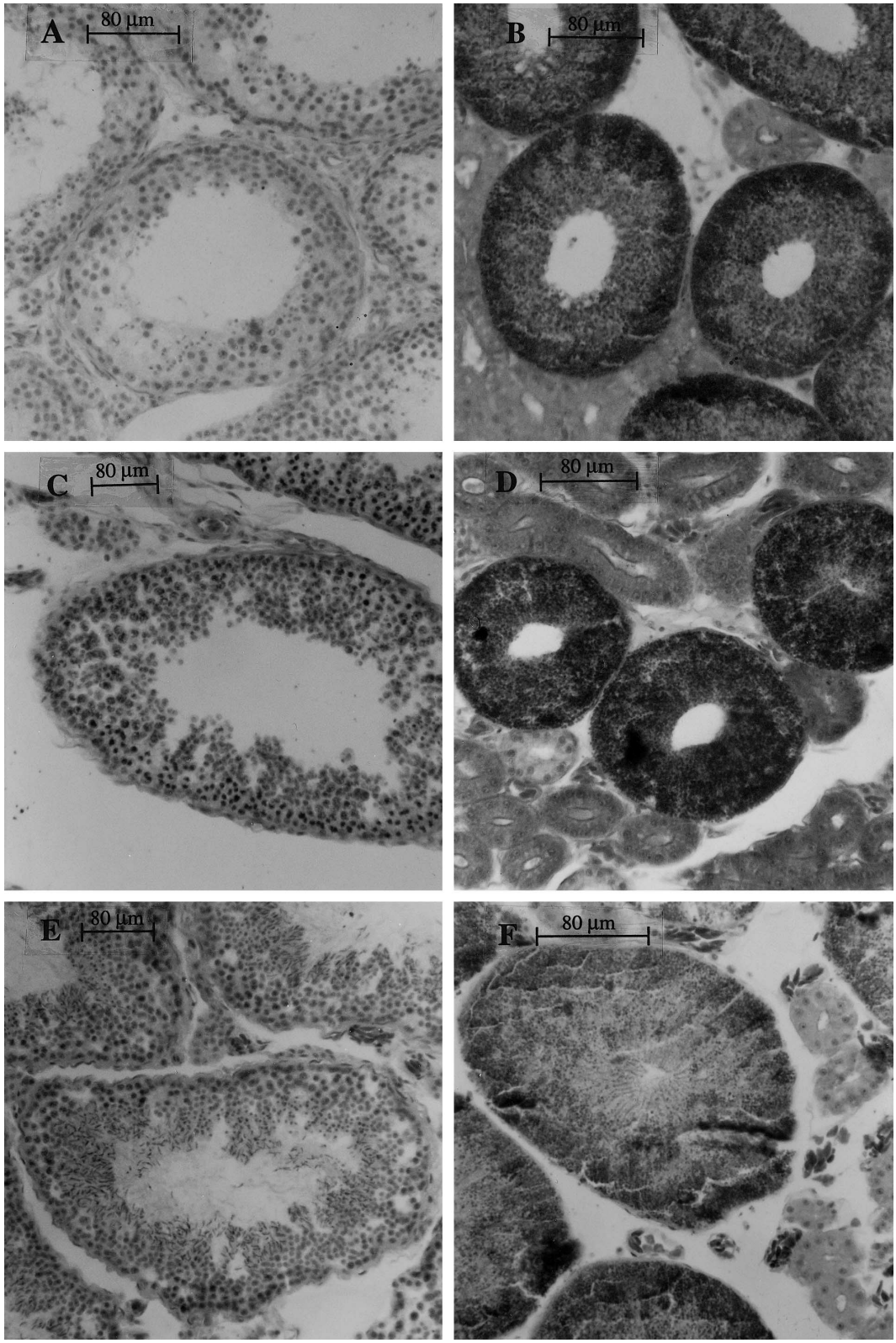
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TABLE 1. Means, standard deviations, and sample sizes (below) for adult *Crotalus viridis helleri* and *Crotalus viridis oreganus*: SVL = snout–vent length (mm), STD = seminiferous tubule diameter (μm), SSK = sexual segment of the kidney diameter (μm). Early season is March and April, Middle season is May and June, and Late season is July to September.

	Early season	Middle season	Late season
SVL	930 ± 126	856 ± 186	917 ± 128
N	12	12	11
STD	194 ± 23	203 ± 61	288 ± 23
N	8	12	11
SSK	189 ± 36	150 ± 16	183 ± 20
N	12	12	11

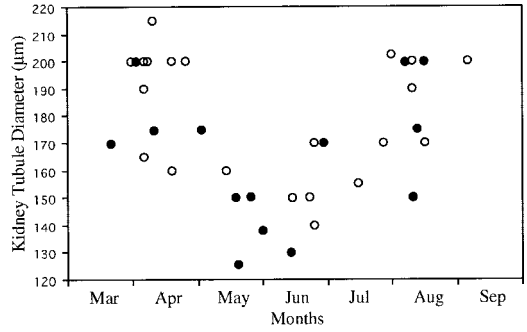

FIG. 4. Diameters of the sexual segment of the kidney of *Crotalus viridis helleri* (dots) and *Crotalus viridis oreganus* (circles). Each point represents the mean of 12 measurements per snake.

oreganus and *C. v. helleri*. The mean SVL, STD, and SSK of *C. v. oreganus* and *C. v. helleri* were compared for the early, middle, and late season. In all of these comparisons the means of SVL and SSK, and two of the three comparisons of the STD were not significantly different between the two taxa. The only significant difference was the mean of the STD in early season ($Z = -2.2$, $P = 0.025$). This difference may be an artifact of small sample sizes and the cut-off dates for the comparisons. The sample size for this period is five for *C. v. helleri* and three for *C. v. oreganus* and the relative diameters are consistent when compared to those from the other two seasons (Fig. 1). Also, if the early May snake (collected 2 May) is included in the analysis, the samples are not significantly different ($Z = -1.2$, $P = 0.22$). For these reasons, the data for the two taxa appear to be similar and are combined in the analysis of seasonal differences in the STD and SSK measurements.

Mean SVLs were not significantly different ($H = 1.3$, $P = 0.5$, Table 1) by season. Mean STDs were significantly different ($H = 16$, $P = 0.004$, Table 1) by season, with the late season being larger than the early and middle seasons (late vs early, $Z = -3.6$, $P = 0.003$; late vs middle $Z = -3.2$, $P = 0.004$; Table 1; Fig. 1). The mean STD was not significantly different between the early and middle season ($Z = -0.3$, $P = 0.76$). The stage of spermatogenesis is presented in Figure 2. Only three snakes had seminiferous tubules containing only spermatogonia and Sertoli cells. These snakes were from March to mid-April. The majority of the snakes examined from April ($N = 5$), May ($N = 6$, Fig. 3A) and June ($N = 4$, Fig. 3C) contained primary and secondary spermatocytes (Fig. 3A). Sperm were first seen in the tubules in the second half of June and all snakes in July through September (Fig. 3E) contained sperm in the seminiferous tubules. In the only September specimen examined, the number of spermatocytes in the tubule was much reduced compared to July and August indicating that spermatogenesis was ending. Spermiation occurred from late June through September. The vas deferens at the level of the kidney contained sperm throughout the active season in all but one snake, a 900 mm snout–vent length (SVL) *C. v. helleri* from May. The smallest snake undergoing spermatogenesis or containing sperm in the vas deferens was 625 mm SVL. The largest male examined was 1210 mm SVL.

Mean diameters of the SSK were significantly different ($H = 16$, $P = 0.0003$, Table 1) by season. Mean diameter of the SSK was significantly higher in early and late seasons compared to the middle season (early versus middle, $Z = -3.6$, $P = 0.003$; late versus middle, $Z = -3.6$, $P = 0.0003$; Table 1; Fig. 4). The diameter of the SSK was not significantly different between the early and late season ($Z = -0.6$, $P = 0.52$). The majority of snakes had basophilic granules in the SSK tubules (Fig. 3).

The seasonal cycles of all reproductive parameters of *C. v. oreganus* and *C. v. helleri* are very similar; thus for the discussion these subspecies will be referred to as the Pacific populations. Comparisons to other reproductive cycles will be limited to other temperate zone snakes.

The seasonal cycle of spermatogenesis and STD of the Pacific subspecies is similar to *Crotalus v. viridis* (Aldridge 1979, 1993), *C. horridus* (Aldridge and Brown, 1995), and *Agkistrodon piscivorus* (Johnson et al., 1982). This summer spermatogenic pattern is also similar to that of most temperate zone colubrids (Saint Girons, 1982). A very different seasonal pattern of spermatogenesis occurs in the vipers, *Vipera aspis* and *Vipera berus* (Saint Girons, 1982). In these species, spermatogenesis begins in the summer and continues into the spring. The two species differ in the occurrence of spermiogenesis. In *V. aspis*, a species that has both a summer and spring mating season, spermiogenesis occurs in the summer and spring, whereas *V. berus*, a species with only a spring mating season, spermiogenesis occurs only in the spring. Thus, in these species, the occurrence of spermiogenesis corresponds to the mating season. Difference in the spermatogenic cycles between pitvipers and vipers cannot be explained solely by the age of sperm at fertilization. In female *V. aspis* that produce offspring from sperm obtained from summer matings, age of sperm at fertilization is similar to pitvipers.

Sperm were present in the vas deferens throughout the year in the Pacific subspecies, a pattern similar to that seen in *C. v. viridis* (Aldridge, 1993) and other populations of *C. v. oreganus* (Diller and Wallace, 1984). As reported in *C. v. viridis*, the age of the sperm used

during copulation is unknown. The sperm used in the summer or spring copulations could have been produced one or two years previously (Aldridge, 1993).

In the Pacific subspecies, the SSK appears to have a bimodal cycle, with the tubules hypertrophied in the Late Season and the Early Season (Table 1). This bimodal cycle may be an artifact of sampling since no measurements are available from winter. The SSK may be hypertrophied throughout the winter, similar to *Natrix maura* and *V. aspis* (Saint Girons, 1982). The mating season of *N. maura* and *V. aspis* is similar to the Pacific populations of *C. viridis*, in that all of these taxa have both a summer and spring mating season.

The diameters of the SSK (Fig. 4) show considerable variation through the active season. These differences may be the result of differences in foraging success of the individuals. Recent unpublished work in my lab on *Nerodia rhombifer* and *Boiga irregularis* has shown that snakes with low coelomic fat reserves have smaller SSK diameters.

The seasonal cycle of the SSK of the Pacific subspecies of *C. viridis* is similar to *A. piscivorus* (Johnson et al., 1982) and most colubrid snakes (Saint Girons, 1982). In these species, the SSK is hypertrophied in the summer and again in the spring. The cycle is different from *C. v viridis* in that the SSK is hypertrophied only in the summer and parallels the hypertrophy of the testis (Aldridge, 1993).

The season of hypertrophy of the SSK in *C. v viridis* and the Pacific subspecies corresponds to the mating seasons. Accounts of mating in *C. v viridis* in the wild indicate that the mating season occurs almost exclusively in the summer, specifically, late July to early September (Aldridge, 1993). In addition to mating, male-male combat appears to be restricted to the season of SSK hypertrophy (Klauber, 1972; Hayes et al., 1992). In the Pacific subspecies a clearly different seasonal pattern of mating and male-male combat activity has been reported. In these taxa, mating occurs in the summer (*C. v oreganus*, Macartney and Gregory, 1988; *C. v helleri*, Klauber, 1972) and again in the spring (*C. v oreganus*, Fitch and Glading, 1947) and, as in *C. v viridis*, the occurrence of male-male combat behavior corresponds to the mating periods. Male–male combat in *C. v oreganus* has been reported in the summer and spring (Klauber, 1972; Hersek et al., 1992).

In summary, the spermatogenic cycle of the Pacific subspecies of *Crotalus viridis* is similar to *C. v viridis*. The seasonal cycle of SSK, however, is remarkably different. In *C. v viridis*, the SSK has a single period of hypertrophy corresponding to the summer mating period. The SSK in the Pacific subspecies is hypertrophied in the summer, perhaps also through the winter, and in the spring. The mating season and male–male combat in the Pacific subspecies occurs during summer and spring corresponding to the hypertrophy of the SSK.

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APPENDIX 1

Specimens Examined

All specimens examined were from the Museum of Vertebrate Zoology (MVZ), University of California, Berkeley, California, the Natural History Museum of Los Angeles County (LACM), California, and the museum at Arizona State University (ASU), Arizona, USA. Unless noted otherwise, all localities of specimens are from California, USA.

Crotalus viridis oreganus

MVZ: 3818, San Luis Obispo; 10537, San Mateo; 14597, Trinity; 17584, Mendocino; 18407, Siskiyou; 21379, 21381, San Benito; 21574, San Mateo; 28214, 28216, 28217, Stanislaus; 34824, Tuolumne; 42653, 42662, 42669, Madera; 92684, 92685, Shasta; 191367, San Joaquin; 193427, 193428, Siskiyou; 29238, Grant (Oregon).

Crotalus viridis helleri

MVZ: 391, 55780, San Diego; 33588, 34643, 35357, Santa Barbara; 35427, Ventura; 55780, San Diego. LACM: 3074, 20083, 20088, 28029, 28031, 59179, Los Angeles. ASU; 30984, Los Angeles.

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Cold-Tolerance of Hatchling Painted Turtles (*Chrysemys picta bellii*) from the Southern Limit of Distribution

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Painted turtles *(Chrysemys picta*) have a natural history unlike that of other chelonians from the northern United States and southern Canada. Although neonates of other freshwater turtles usually emerge from their subterranean nests in late summer or autumn and move to nearby marshes, lakes, or streams to spend their first winter, hatchling painted turtles typically remain inside their shallow (8–14 cm) nests throughout their first winter and do not emerge above ground until the following spring (Ernst et al., 1994). This behavior commonly causes neonatal painted turtles from Nebraska (Packard, 1997; Packard et al., 1997a), northern Illinois (Weisrock and Janzen, 1999), and New Jersey (DePari, 1996) northward to the limit of distribution in southern Canada (Storey et al., 1988) to be exposed during winter to ice and cold, with temperatures in some nests going below −10°C. Many hatchlings withstand such extremes and emerge from their nests when the ground thaws in the spring (Storey et al., 1988; DePari, 1996; Packard, 1997; Packard et al., 1997a; Weisrock and Janzen, 1999).

Hatchling painted turtles from northern populations withstand exposure to ice and cold by remaining unfrozen at temperatures below the equilibrium freezing point for their body fluids (Packard and Packard, 2001). This supercooled state occurs (1) because the body fluids of hatchlings usually do not contain the necessary organizing sites (= nucleating agents) to initiate freezing at temperatures above −15°C (Costanzo et al., 1998, 2000; Packard and Packard, 1999) and (2) because the integument of the turtles resists the penetration of ice crystals into body compartments from frozen soil (i.e., ''inoculation''; Costanzo et al., 2000; Willard et al., 2000). In the absence of a suitable organizing site to promote a change in phase from liquid to solid, the body fluids of hatchlings remain in an unfrozen, liquid state (Dorsey, 1948; Franks, 1985). Supercooled solutions are quite stable at subzero temperatures above −20°C (Dorsey, 1948), so turtles can remain unfrozen for extended periods during winter (Packard and Packard, 1997; Hartley et al., 2000).

The preceding generalizations are based, however, on studies of hatchling painted turtles from higher latitudes. Little is known about the tolerance for cold in animals from populations at lower latitudes, yet such information is key to reconstructing the evolutionary history of painted turtles (Bleakney, 1958; Ultsch et al., 2001) and to understanding the post-Pleistocene expansion in the range of the species (Holman and Andrews, 1994). Accordingly, we report here the results of three experiments on cold-tolerance of hatchling painted turtles from a disjunct population near the southern limit of distribution for the species in the Rio Grande Valley of central New Mexico. We find that neonates from New Mexico have the same means and capacity as hatchlings from North Dakota for dealing with the challenges of ice and cold, despite the fact that winters at the New Mexico site are likely to be substantially milder than those in North Dakota.

Animals.—We captured four gravid painted turtles in late May 2000 near San Marcial, in the Rio Grande Valley south of the Bosque del Apache National Wildlife Refuge, Socorro County, New Mexico. Animals from this population currently are assigned to the subspecies *bellii* (see Ultsch et al., 2001), which comprises animals that presumably are descended from turtles occupying a southwestern refugium during Pleistocene glaciation (Bleakney, 1958). We injected the turtles with oxytocin to induce them to lay their clutches of fully formed eggs, after which the eggs were packed in damp sand, transported to Iowa State University, and incubated to hatching on moist vermiculite (water potential = −150 kPa) at 28.3°C. Twenty-two hatchlings from these clutches later were shipped by air express to Colorado State University where experiments on cold-tolerance were performed.

When the turtles arrived in Colorado, they were placed immediately into a darkened environmental chamber at 20°C. Temperature in the chamber was reduced in steps of 1–2°C every two days to 4°C (nom-

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inal temperature), which then was maintained until the animals were used in the following studies.

Experiment 1.—Six turtles (representing all four clutches) were dried carefully and cleaned with a small paint brush, after which a copper/constantan thermocouple (26 gauge wire) was glued to the carapace of each animal with epoxy resin. The turtles were placed individually into pint-volume canning jars where they rested on a surface of dry styrofoam to prevent them from contacting ice and possibly being inoculated. The closed jars were placed into a Percival environmental chamber set at 2°C, and the thermocouples were attached to a Campbell CR-10 datalogger that recorded temperature every 10 min.

We programmed the controller for the chamber to reduce temperature linearly by 1°C/day. On reaching a minimum of −20°C, the temperature was immediately returned to 2°C. Turtles were removed from the jars the following morning, placed into paper cups containing a small amount of water and given two days to recover. The hatchlings then were judged to be alive or dead on the basis of their spontaneous activity, their responses to tactile stimuli, the appearance of their eyes (wide open and focused vs fully closed or partially closed and vacant), and their general level of alertness.

We downloaded data from the datalogger to a PC and then constructed a temperature profile for each turtle. These temperature profiles were examined for the presence of freezing exotherms (i.e., for the abrupt increases in temperature resulting from the release of latent heat of fusion by water changing phase from liquid to solid). The temperature on the carapace of an animal immediately before the appearance of a freezing exotherm was taken to be the limit of supercooling for the turtle.

One turtle in this test froze spontaneously at the relatively high temperature of −12.0°C, but values for the limit of supercooling for the other five animals were clustered between −17.1°C and −18.6°C. The arithmetic mean for the limit of supercooling was −17.0°C (SD = 2.5°) for the six turtles in our sample, and the median was −17.8°C. None of the hatchlings survived the treatment.

Experiment 2.—When none of the turtles survived the preceding experiment, we set out to determine whether neonatal animals are able to withstand exposure to moderate subzero temperatures above their limit for supercooling. Accordingly, the six hatchlings (again representing all four clutches) in this second experiment were treated in the same way as animals in the preceding experiment, except that temperature in the chamber was lowered only to −8.5°C. This minimum was maintained for 24 h, after which temperature was returned to 2°C and the condition of the animals (i.e., alive or dead) was assessed. Temperature profiles for the turtles again were examined for the presence of freezing exotherms.

Our protocol caused the turtles to be exposed for 8 days to temperatures below the equilibrium freezing point for their body fluids (approximately −0.7°C; Storey et al., 1991; Packard and Packard, 1995; Costanzo et al., 2000) and for the last 24 h to minima averaging −8.7°C (SD = 0.2°; range, −8.5°C to −8.9°C). The variation in minima recorded in different jars is merely a reflection of the fact that environmental chambers seldom maintain uniform conditions throughout their interior (Measures et al., 1973). None of the turtles froze during the course of this experiment, and all the hatchlings survived their exposure.

Experiment 3.—The remaining 10 turtles (representing all four clutches) were prepared for study as described previously, but for this experiment the hatchlings were placed individually into artificial nests constructed in jars of damp, loamy sand (water content, 25 g/100 g dry soil; water potential, approximately −50 kPa as estimated by thermocouple hygrometry). Soil was tamped gently into spaces around each hatchling to maximize its contact with the substratum and thereby maximize the probability that the turtle would be inoculated when water in the soil was subsequently caused to freeze (Salt, 1963).

The closed jars were placed into the Percival environmental chamber, which was set to bring temperature in the jars to approximately −0.4°C. This temperature is below the equilibrium freezing point for water in moist soils (Bodman and Day, 1943) but above that for body fluids of baby painted turtles (−0.7°C; Storey et al., 1991; Packard and Packard, 1995; Costanzo et al., 2000). Each jar then was opened; a few pieces of shaved ice were placed onto the surface of the soil; and the jar was closed and quickly placed back into the environmental chamber. Supercooled water in the soil began to freeze immediately, as was indicated by a sudden increase in temperature (i.e., by exotherms) in every jar to approximately 0°C (Fig. 1). The temperature in the chamber was held at the nominal level of −0.4°C for four days so that water in the soil could freeze to an equilibrium.

After four days, temperature in the chamber was lowered linearly at a rate of 1°C/day to a minimum near −4.5°C. This minimum was maintained for seven days before temperature in the chamber was reset to 2°C and the jars (and turtles) were allowed to rewarm. The turtles then were removed and their condition (alive or dead) was assessed from their appearance and behavior. The minimum temperature and the duration of exposure used here were the same as were used in an earlier investigation of neonatal *bellii* from northern North Dakota (Packard et al., 1997b), thereby to enable us to compare responses by animals from a southerly population with those of turtles from near the northern limit of distribution. Later we downloaded data from the datalogger to a PC and constructed a temperature profile for each jar (i.e., for the turtle and surrounding soil).

Temperature in the jars averaged −0.5°C (SD = 0.1°C; range, −0.2°C to −0.6°C) after water in the soil had frozen to a thermal equilibrium (Fig. 1), so none of the animals was at risk of freezing at this early point in the experiment (because temperature in all the jars was above the equilibrium freezing point for body fluids of the turtles). Temperature then was reduced to a minimum averaging −4.5°C (SD = 0.2°C; range, −4.2°C to −4.7°C), which was maintained for the requisite seven days. Temperature profiles revealed that three turtles froze during their exposure (Fig. 1A) but that remaining hatchlings remained unfrozen (Fig. 1B). In all instances where an animal froze, ice began to form in its body fluids only after the turtle already had been in contact with ice and at temperatures below the equilibrium freezing point for

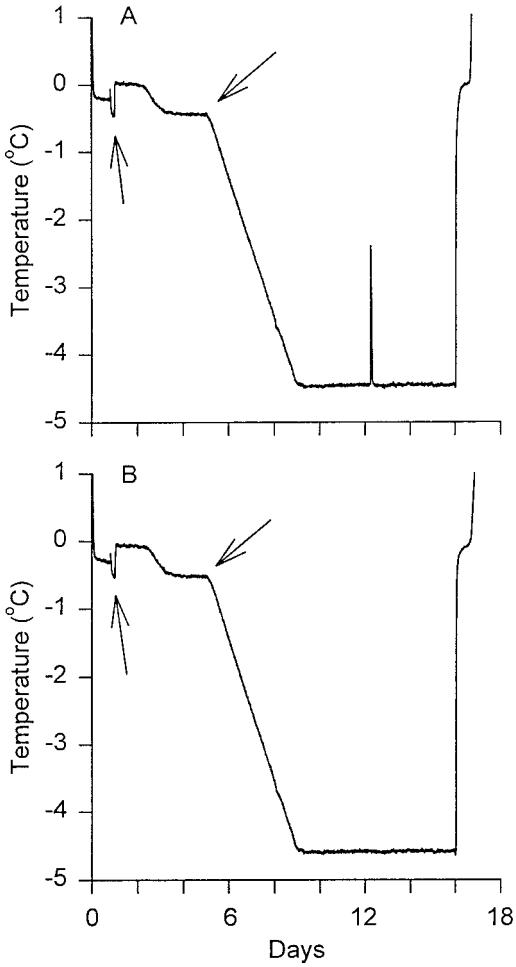

FIG. 1. Temperature profiles for hatchling painted turtles confined in artificial nests in jars of damp, loamy sand. The left-hand arrow in each panel identifies the time at which ice was added to the jar to induce freezing of supercooled water in the soil. The right-hand arrow identifies the time at which temperature began to decline linearly at the rate of 1°C/day. (A) Profile for a turtle that froze after eight days in contact with ice and at temperatures below the equilibrium freezing point for body fluids; the spike in temperature on day 13 of the test is a freezing exotherm for the hatchling. (B) Profile for a turtle that remained unfrozen for the duration of its exposure.

the body fluids for 4–8 days (Fig. 1A). Frozen turtles were dead at the end of the experiment, but all the unfrozen animals were alive (Table 1).

Discussion.—Turtles in the first experiment were prevented from making contact with crystals of ice that might have penetrated their integument and caused their body fluids to freeze. Thus, freezing of these animals must have been initiated by heterogeneous nucleation (i.e., by nucleation caused by appropriately configured contaminants or inclusions), because homogeneous nucleation (i.e., spontaneous formation of suitable organizing sites by water molecules themselves) rarely occurs at temperatures above −20°C (Dorsey, 1948; Franks, 1985). Also, the nucleating agents in question were not overly efficient, because the animals typically did not freeze spontaneously until their body temperature was near −17°C. This value is indistinguishable from those reported for hatchling *bellii* from more northerly populations in Nebraska (Packard and Packard, 1999; Costanzo et al., 2000).

TABLE 1. Survival by hatchling painted turtles confined in artificial nests in jars of damp, loamy sand and then exposed to −4.5°C for seven days. A freezing exotherm appeared in the temperature profile for each of the turtles that is said to have frozen, but no exotherm was detected in the profile for any other animal. Data for hatchlings from New Mexico are from the current study; those for neonates from North Dakota were taken from Packard et al. (1997b). The frequencies for freezing by animals in the two studies could not be distinguished statistically (Fisher's Exact Test, $P = 1.0$).

Turtle froze	New Mexico		North Dakota	
	Alive	Dead	Alive	Dead
Yes	0	3	0	8
No	7	0	24	0

Turtles in the second experiment also were prevented from making contact with crystals of ice that might have penetrated their integument and caused their body fluids to freeze. None of these animals froze, and all survived their exposure to temperatures near −8.5°C. These findings reflect a level of cold-tolerance in hatchlings from central New Mexico similar to that of neonates from more northerly populations of *bellii* in Nebraska (Packard and Packard, 1999), Minnesota (Packard et al., 1999), and North Dakota (Packard et al., 1997b).

The three turtles that froze during their exposure to subzero temperatures in the third experiment presumably were caused to freeze by ice penetrating into body compartments from the surrounding soil, because none of the animals was exposed to a temperature low enough to elicit spontaneous freezing of its body fluids by heterogeneous nucleation. However, the integument of these animals afforded some resistance to the inward growth of ice crystals, because the turtles did not freeze until they had been in contact with ice (and at temperatures below the equilibrium freezing point for their body fluids) for several days. Additionally, the other seven turtles in this third experiment remained unfrozen for the duration of their exposure. Had the integument of the 10 animals in this test not resisted the penetration of ice into body compartments, the turtles surely would have frozen soon after their body temperature went below the equilibrium freezing point, much as occurs when frogs are caused to freeze by inoculation (Layne et al., 1990; Layne, 1991; Costanzo et al., 1999). A cutaneous barrier to penetration of ice also is characteristic of hatchling painted turtles from northerly populations of *bellii* (Packard et al., 1997b, 1999; Costanzo et al.,

2000; Willard et al., 2000); indeed, turtles from New Mexico had the same resistance to inoculation as animals from North Dakota (Table 1).

Finally, the animals that survived the third experiment were the ones that remained unfrozen, and the turtles that froze were the ones that died (Table 1; $P = 0.008$ by Fisher's Exact Test). Virtually identical results again were reported for hatchling painted turtles from populations of *bellii* in Nebraska (Packard and Packard, 1997), North Dakota (Packard et al., 1997b), and Minnesota (Packard et al., 1999).

Thus, neonates from the southern limit of distribution for *Chrysemys picta bellii* have a level of cold-tolerance that is indistinguishable from that of hatchlings from populations at the northern limit of distribution. Animals from northern and southern populations are able to resist the penetration of ice into body compartments from frozen soil, and they also have similar limits for supercooling. We do not know, however, whether the resistance to ice and cold manifested by hatchlings from New Mexico is an adaptation to conditions encountered in nests during winter or whether it derives from a suite of characters preadapting neonates for overwintering in nests at higher latitudes.

Acknowledgments.—Our procedures were considered and approved by the Committee for Care and Use of Animals in Research at Iowa State University (protocol 12-9-4382-1-J) and by the Committee for Animal Care and Use at Colorado State University (protocol 00-101A-01). Female turtles were collected and handled under authority of permit 3040 from the New Mexico Department of Game and Fish. The research was supported in part by a Grant-in-Aid-of-Research from the Society of the Sigma Xi (to CM), by a predoctoral fellowship from the National Science Foundation (to CM), and by grant IBN-9612562 from the National Science Foundation (to GCP). We also thank M. Knutzen for helping to care for the eggs.

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The Breaking of Diapause in Embryonic Broad-Shell River Turtles (*Chelodina expansa*)

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The Australian broad-shelled turtle (*Chelodina expansa*) typically constructs its nests during the Austral autumn or early winter when soil temperatures are decreasing (Goode and Russell, 1968; Legler, 1985; Booth, 1998). Eggs laid in early autumn may experience warm temperatures for a month or two before soil temperature drops below 20°C, eggs laid in winter experience cool soil temperatures immediately, and eggs laid in rare late season nests experience warm temperatures (> 20°C) throughout incubation (Booth, 1998). Embryos of *C. expansa* normally experience two periods of developmental diapause during ontogeny (Booth, 2000). The first is a preovipositional diapause (primary diapause) termed ''extension of preovipositional arrest'' by Ewert and Wilson (1996) that is common to all turtles (Ewert 1985, 1991), and in *C. expansa* this may extend for up to six weeks after oviposition (Booth, 2000). Once primary diapause is broken and the white-patch has developed to cover half to three-quarters of the eggshell, embryos invariably enter a second diapause period termed ''embryonic diapause'' by Ewert and Wilson (1996).

Embryos of *C. expansa* have an exceptionally long incubation period not only because embryonic development is inherently slow (Goode and Russell, 1968; Legler 1985) but also because embryos enter a secondary diapause stage (Booth, 1998, 2000). Other turtle species are reported to have extended incubation periods because of developmental attest late in incubation (Ewert, 1985, 1991; Webb et al., 1986), but in *C. expansa* patterns of oxygen consumption indicate that development is continuos during the latter phase of incubation and that developmental arrest is confined to early incubation (Booth, 2000). If eggs of *C. expansa* are artificially incubated at a high and constant temperature immediately after oviposition, embryos still enter secondary diapause, but a large proportion fail to break out of this secondary diapause phase and perish (Booth, 2000). The failure to break secondary diapause when incubated at high and constant temperature appears to be a feature of turtle species that experience a secondary diapause period during embryonic development (Ewert 1985, 1991). Those embryos that break diapause do so asynchronously so that eggs from the same clutch hatch over a large period of time (up to 70 days; Booth, 2000). Asynchronous hatching is probably maladaptive in natural nests (Booth, 2000), but embryos in natural nests appear to hatch at a similar time (Booth, 1998). In natural nests of *C. expansa*, both daily and seasonal changes in nest temperature occur (Booth, 1998), so temperature is a likely cue for the synchronous breaking of secondary diapause in this species. Indeed changes in temperature appear to break arrested development in other turtle species (Ewert, 1991; Ewert and Wilson, 1996). In a closely related species *Chelodina rugosa*, which has the unusually habit of depositing its eggs underwater in drying swamps, the stimulus for breaking primary diapause is drying of mud which then allows oxygen to enter the egg (Kennett et al. 1993). However, embryos of *C. rugosa* have never been reported to enter secondary diapause during embryonic development. I artificially incubated eggs of *C. expansa* under three different thermal regimes in order to investigate the role change in temperature has in breaking secondary diapause.

I collected two clutches of eggs of *C. expansa* immediately after natural ovipostion on 26 May 2000. Eggs were transported to the laboratory, rinsed briefly in tap water to remove soil adhering to the eggshell, and weighed. Eggs had the clutch and egg number marked on the eggshell with graphite pencil, and eggs from each clutch were evenly distributed across three plastic incubation boxes. Eggs were incubated half-buried in vermiculite with a water potential of −150 kPa and sealed in boxes with a loose-fitting lid. Temperature data loggers that recorded temperature twice per hour were placed in boxes at this time. Eggs and boxes were weighed periodically throughout incubation, and water lost from the vermiculite was replaced to ensure relatively stable water potential throughout incubation (Packard et al., 1981). Each of the boxes was assigned to one of three treatments (Fig. 1). In the first treatment, which was designed to imitate eggs laid in early autumn, eggs were incubated at 25°C for 30 days, transferred to 18°C for 67 days and then incubated until hatching at 28°C. In the second treatment, which was designed to imitate eggs laid during winter, eggs were incubated for 97 days at 18°C then incubated until hatching at 28°C. In the third treatment, which was also designed to imitate eggs laid during winter, eggs were incubated for 97 days at 18°C, then

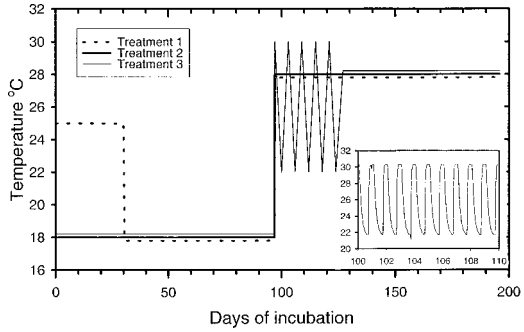

FIG. 1. The three thermal treatments used in experiments. Insert is a 10-day sample of logged temperature during the 30-day period of daily cyclic temperature fluctuations from treatment 3.

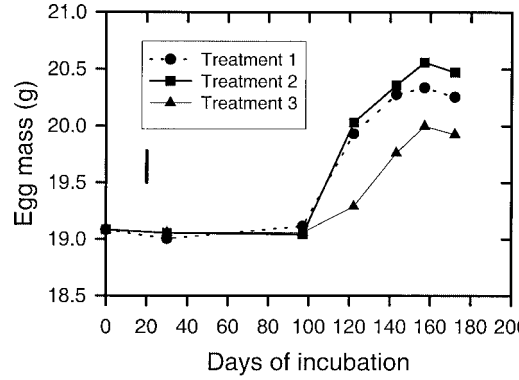

FIG. 2. Egg mass changes during incubation in the three thermal treatments. Plotted values are means adjusted by ANCOVA procedure with initial egg mass as the covariate (Packard and Boardman 1988). Vertical bar is the critical range determined by Newan-Keuls test at $P = 0.05$.

transferred to an incubator whose temperature fluctuated cyclically between 22°C and 30°C each day for 30 days, and then incubated at 28°C until hatching occurred. Around the time of hatching, boxes were examined six times per day so that day of hatching could be recorded. Once hatched, hatchlings had adhering vermiculite brushed free and were placed in plastic vials marked with their egg number. The vials were stored in the 28°C incubator for a further two days to allow complete absorption of residual yolk into the abdomen, and to allow the carapace to assume its normal shape. After this storage period hatchlings were weighed and had their carapace length and width, plastron length and width, and head width measured with calipers.

Repeat measures anaylsis of covariance (ANCOVA) with initial egg mass as the covariate was used to test for differences in egg mass between treatments throughout incubation. ANCOVA with initial egg mass as the covariate was use to test for treatment differences in hatchling parameters as variations in incubation period may well affect the amount of energy utilized during embryonic development and this may be reflected in hatchling size. Incubation time variance was significantly different between clutches and treatments so nonparametric Mann-Whitney *U*-tests were used to test for clutch and treatment effects in these cases. Statistical analysis was performed using Statistica® software, and I assume statistical significance if $P < 0.05$.

Ideally, thermal treatments should have been replicated to avoid the possibility of confounding treatment effects with incubator effects (Packard and Packard, 1993). Unfortunately a lack of incubator resources and the small number of eggs available for each treatment cell precluded such a design. However, in turtle egg incubation experiments, clutch and treatment usually have such large effects that they overwhelm any other effects (Packard and Packard, 1993). Hence, the risk of drawing incorrect conclusions by confounding treatment effects with incubator and box effects is small.

One clutch contained 13 eggs and the other 20 eggs. All eggs in all treatments ended primary diapause as indicated by the appearance of ''white patches'' on their dorsal surfaces (Ewert, 1985, 1991; Ewert and Wilson, 1996; Booth, 2000) during the first 32 days of the experiment. The white-patch ceased to grow once it covered one-half to two-thirds of the eggshell's surface in all eggs indicating the start of the secondary diapause phase of development (Ewert and Wilson, 1996; Booth, 2000). Once eggs were transferred to

TABLE 1. Hatchling parameters of eggs incubated under the three different thermal regimes. For hatchling parameters reported means have been adjusted by ANCOVA procedure with initial egg mass as the covariate. Data reported as means ± standard errors. Statistical comparison by ANCOVA.

Parameter	Treatment 1	Treatment 2	Treatment 3	Probability
Sample size	12	11	10	
Initial mass (g)	19.16 ± 0.80	18.82 ± 0.70	19.27 ± 0.76	$P = 0.911$
Hatchling mass (g)	12.70 ± 0.54	12.88 ± 0.49	12.68 ± 0.60	$P = 0.493$
Carapace length (mm)	40.7 ± 0.4	40.6 ± 0.3	40.8 ± 0.4	$P = 0.943$
Carapace width (mm)	33.0 ± 0.3	33.1 ± 0.3	33.5 ± 0.3	$P = 0.239$
Plastron length (mm)	29.9 ± 0.4	30.2 ± 0.4	30.0 ± 0.4	$P = 0.499$
Plastron width (mm)	18.7 ± 0.2	18.8 ± 0.3	18.1 ± 0.2	$P = 0.088$
Head width (mm)	11.6 ± 0.1	11.6 ± 0.1	11.8 ± 0.1	$P = 0.184$

TABLE 2. Effect of clutch and incubation temperature on days until primary diapause was broken. Means ± standard error (sample size). Statistical comparisons made with Mann-Whitney *U*-tests.

	18 °C	25 °C	Temperature effect?
Clutch 1	24.5 ± 2.9 (8)	12.2 ± 0.6 (5)	Yes ($P = 0.019$)
Clutch 2	5.8 ± 0.2 (13)	6.1 ± 1.0 (7)	No ($P = 0.812$)
Clutch effect?	Yes ($P < 0.001$)	Yes ($P = 0.007$)	

warmer temperatures on day 97, the white-patch began growing again indicating the breaking of secondary diapause (Ewert and Wilson, 1996; Booth, 2000). The white-patch continued to grow until it covered the entire surface of the eggshell. All eggs in all treatments hatched successfully.

During the first 97 days of incubation, there was no significant change in egg mass in any treatment (Fig. 2). However, once eggs were transferred to warmer temperatures on day 97, secondary diapause was broken, and eggs in all treatments gained mass (Fig. 2). The increases in egg mass of eggs in treatments 1 and 2 were similar, but the mass gained by eggs in treatment 3 was significantly less during the 30 days when the temperature fluctuated cyclically on a daily basis (Fig. 2). All changes in egg mass during incubation were assumed to be caused by the exchange of water (Packard et al., 1981). During the period of secondary diapause, there was no net exchange of water across the eggshell as indicated by no change in egg mass during this time. However, once secondary diapause was broken, all eggs absorbed water. Eggs in treatment 3 absorbed significantly less water during the time that daily fluctuations in temperature occurred compared to eggs incubated at constant temperature. The exchange of water between eggs and the incubation medium is a complicated process involving both liquid water and water vapor exchange (Packard et al., 1981; Ackerman, 1991; Packard, 1991). The exchange of water vapor is very sensitive to small temperature gradients across the eggshell (Ackerman, 1991; Packard, 1991), so the difference in the amount of water exchanged by eggs in the fluctuating and constant temperature regimes is most probably caused by differences in the amount of water vapor exchanged. Because temperature in natural nests fluctuates daily (Booth, 1998), the amount of water exchanged by eggs in natural nests may be considerably less that that of eggs incubated artificially at constant temperature.

Incubation treatment had no significant effect on any of the measured hatchling parameters (Table 1), indicating that hatchlings from all treatments were of similar quality.

Clutch of origin had no effect on incubation period ($P = 0.587$), but hatchlings from treatment 3 took longer to hatch than either treatments 1 and 2 (Table 3) and hatchlings from treatment 2 had a smaller range and variance in hatchling times compared to treatments 1 and 3 (Table 3). A Mann-Whitney *U*-test indicated a clutch effect ($P < 0.001$) on the time until primary diapause was broken with eggs from clutch 1 having longer primary diapause periods than eggs from clutch 2 (Table 2). Temperature also affected primary diapause period in clutch 1 but did not affect primary diapause period in clutch 2 (Table 2). Clutch effects have previously been described for various aspects of incubation of turtle eggs (Packard and Packard, 1993). Because eggs are held in primary diapause within the uterus until suitable laying conditions occur (usually heavy rainfall; Booth, 1998, 2000), it is possible that interclutch variation in the postoviposition primary diapause period is related to the amount of time eggs are held in the uterus before being laid. Eggs held for longer periods may have a shorter postoviposition diapause period than eggs held for long periods of time. Eggs incubated at 18°C were able to break primary diapause and develop to the secondary diapause stage despite the fact that once secondary diapause is broken, a temperature of 18°C is too low to allow embryonic development to proceed further (Booth, 1998). The ability to continue to develop to the secondary diapause stage at low temperature would allow eggs oviposited latter in the laying season to develop to the secondary diapause stage despite low soil temperatures. Thus there does not appear to be any advantage to laying eggs early in the season when soil temperatures are still at a level when continuos embryonic development is possible, because they do not hatch earlier than eggs laid later in the season. Indeed, early laid eggs may be at a disadvantage as they are exposed to the vagaries of the nesting environment for longer periods of time.

TABLE 3. Effect of temperature treatment on incubation time. Data for eggs continuously incubated at 28 °C are from two clutches in a previous study (Booth 2000). Statistical comparisons between treatments within the current study made with Mann-Whitney *U*-tests because Leven's test for homogeneity of variance indicated unequal variance between treatments.

Parameter	28 °C	Treatment 1	Treatment 2	Treatment 3	Probability
Sample size	10	12	11	10	
Mean (d)	164.2	184.0	181.4	192.2	$P < 0.001$
Variance (d)	6.3	3.2	0.9	3.5	$P < 0.001$
Range (d)	150–171	181–191	180–183	187–196	

Incubation at 25°C for 30 days before cooling to 18°C had no effect on overall incubation time suggesting that embryos entered but failed to break out of secondary diapause during the initial phase of incubation at 25°C. Despite differences in the time to break primary diapause, all eggs incubated continuously at 28°C after being held at 18°C hatched at similar times, suggesting that all eggs arrested development during secondary diapause at the same developmental stage. The relatively larger range in incubation times of treatments 1 and 2 compared to treatment 3 probably indicates a larger degree of asynchronous breaking of secondary diapause in the former treatments. However this range (10 days) is far less than the 21 days reported for *C. expansa* eggs incubated continuously at high temperatures immediately after laying (Table 3). This observation suggests that an increase in incubation temperature may be a mechanism by which breaking of secondary diapause is synchronized within a clutch. However, daily fluctuations in temperature did not increase the degree of synchrony of hatching compared to eggs that experienced a single step increase in incubation temperature.

Eggs from treatment 3 in which the incubation temperature cycled for the first 30 days after the temperature was raised from 18°C had significantly longer incubation times compared to eggs that were incubated continuously at 28°C during this time. The average incubation temperature from treatment 3 eggs during the temperature fluctuation period was 25.6°C. Thus the observation that eggs from this treatment had longer incubation times is not surprising as it is well known that reptile eggs have longer incubation times at lower temperatures (Deeming and Ferguson, 1991).

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