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Geographic variation in the long-nosed snake, *Rhinocheilus lecontei* (Colubridae): beyond the subspecies debate

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Scalation, colour pattern, linear and geometric morphometrics were used to quantify geographical differentiation in the long-nosed snake, *Rhinocheilus lecontei,* and to test the hypothesis that all four subspecies are morphologically distinct. Also investigated were potential associations between morphological (scalation, colour pattern, linear measurements) and environmental variables (climate, vegetation, soil). Sexual dimorphism was weakest for geometric and strongest for linear morphometric variables. Morphological variables differed widely in their ability to differentiate subspecies. Linear morphometric variables achieved the most statistically significant pairwise Mahalanobis distances between subspecies, while geometric morphometrics largely failed to differentiate them. Colour pattern showed the strongest and linear morphometrics the weakest correlation with environment. Several characters varied continuously along latitudinal or longitudinal gradients, such that, in some cases, the clines for closely related traits were discordant. No one subspecies was consistently divergent in all analyses, leading to the conclusion that the three mainland subspecies are not sufficiently distinct to warrant separate subspecies status. The island subspecies, though not always statistically distinct, is geographically separate from other populations and differs in characters related to size. Given the small number of specimens available, a decision regarding its taxonomic status (i.e. elevation to species level) is best deferred until additional specimens can be examined and data on molecular variation can be analysed. *Biological Journal of the Linnean Society*, 2004, **83**, 65–85.

ADDITIONAL KEYWORDS: colour pattern – geometric morphometrics – linear morphometrics – scalation – sexual dimorphism – thin-plate splines.

INTRODUCTION

The subspecies concept has been the subject of decades of controversy, primarily due to an unfortunate history of abuse at the hands of vertebrate taxonomists. The naming of subspecies was most popular from the turn of the twentieth century until the 1950s (Mayr, 1982), at which time minor dissimilarities among a few study specimens were enough to warrant subspecific status. Many subspecies descriptions were based on a single specimen or on variation caused by damage or discoloration (Mayr, 1982). Another misuse was to infer allopatric distributions and disjunct patterns of variation where poor geographical sampling masked patterns of smooth clinal differentiation (Montanucci, 1992). In some cases, locations along known clines were arbitrarily chosen as subspecies boundaries (Mayr, 1982; Frost & Hillis, 1990). The subspecies concept was also applied to taxa with traits that varied independently and discordantly, such that distinct morphological entities did not exist (Wilson & Brown, 1953; Inger, 1961). In addition, statistical analyses, rarely included in subspecies descriptions, were usually limited to univariate methods and very seldom investigated sexual dimorphism. In the 1950s, the subspecies concept was subjected to strong criticism (Wilson & Brown, 1953), despite the introduction of a more stringent definition over a decade earlier

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(Mayr, 1942). Thus began a lively dialogue that has endured half a century.

Much of the disagreement on subspecies followed from divergent philosophies about its purpose. For many workers after Darwin (1859), subspecies represented incipient species, while for others subspecies represented patterns of local adaptation (Mayr, 1982). The first interpretation is systematic in nature and the second is taxonomic (Inger, 1961; Mayr, 1982; O'Neill, 1982). Followers of the systematic approach advocated restricting the application of subspecies to populations with strictly allopatric distributions (Mayr, 1982). This criterion of allopatry became problematic when some workers argued that the geographical separation of populations can be a matter of temporal perspective (Wilson & Brown, 1953; Inger, 1961; Van Devender *et al*., 1992). Geographic distributions may fluctuate, causing an allopatric distribution to be transient on a geological time scale. Furthermore, proponents of the evolutionary species concept (Wiley, 1978; Frost & Hillis, 1990) argued that distinct, allopatric subspecies should be elevated to full species. This methodology has been applied extensively in reptiles and amphibians (Collins, 1991; Grismer, 1999) with mixed reactions from herpetologists (Montanucci, 1992; Van Devender *et al*., 1992).

Meanwhile, the taxonomic approach encouraged many of the patterns of abuse cited above, in the name of documenting geographic variation. Efforts to describe patterns of microgeographic variation produced species with as many as 35 trinomials (Chasen, 1940), thereby diluting the significance of the subspecies designation. Inconsistent applications of the subspecies concept due to differences in systematic vs. taxonomic approaches resulted in perceptions of misuse, leading some workers to suggest abandoning the trinomial altogether (Wilson & Brown, 1953; Cracraft, 1983; McKitrick & Zink, 1988). The majority argued, albeit weakly, to keep the subspecies ranking, citing its usefulness in documenting patterns of geographic variation and regions of interest to evolutionary biologists (Monroe, 1982; O'Neill, 1982; Parkes, 1982). Nevertheless, the number of new subspecies descriptions has declined drastically (Collins, 1990, 1992). Most workers have moved beyond the subspecies debate and no longer discuss geographic variation in the context of trinomials. Nevertheless, trinomials remain, and a new generation of systematists is faced with the daunting question of what to do with them. A logical first step is to evaluate the distinctiveness of existing subspecies.

Here, I evaluate the morphological distinctness of the four subspecies of the long-nosed snake, *Rhinocheilus lecontei* (Baird & Girard), using univariate and multivariate analyses of scalation, colour pattern and linear morphometric variables (LMV) and multivariate analyses of geometric morphometric variables (GMV). I chose this species as a case study, because its intraspecific variation lends itself to subspecies classifications that span the gamut from arbitrary designation to full species under the evolutionary species concept (Grismer, 1999). I also explore the ability of traditional linear morphometrics vs. geometric morphometrics to discriminate between subspecies. Finally, I examine the relationship between the morphological and environmental variables using canonical correlation analysis.

Rhinocheilus lecontei

Rhinocheilus lecontei has a widespread distribution in the western United States and northern Mexico. The genus has undergone a number of taxonomic evaluations, the most recent of which was the proposed elevation of an island subspecies to full species status, a decision based on the evolutionary species concept (Grismer, 1999). For the purposes of this study, however, I will maintain the previous taxonomic arrangement of a single species with four subspecies: *antonii* in northern Mexico, *etheridgei* restricted to Isla Cerralvo in the Sea of Cortés, *lecontei* in the north-west portion of the range, and *tessellatus* in the north-east portion (Fig. 1). Its colour pattern features dorsal blotches or saddles that may contact the ventral scales and may be either all black or alternating between black and red. Some spotting or graininess usually occurs laterally due to a colour inversion in the centre of some scales. On a black background, the grains are cream or red in colour, and on a cream or red background, they are black. The rostral scale is enlarged, hence the species' common name, and may angle upward.

The most comprehensive authority on geographic variation in *R. lecontei* is Klauber's (1941) monograph, which recognized three of the current subspecies (*antonii*, *lecontei* and *tessellatus*) and provided detailed descriptions of morphology, scalation, colour pattern and intrasubspecific trends. Klauber (1941) also described a new subspecies, *clarus*, whose distinguishing characteristics were fewer and longer dorsal saddles with very little lateral graininess between the saddles. The sympatric occurrence of this new form with *lecontei*, however, caused some concern (Smith, 1942; Smith & Hensley, 1958), until Shannon & Humphery (1963) discovered both colour morphs in four juveniles hatched from a single clutch. They recommended that *clarus* be considered a morph of *lecontei*, not a distinct subspecies. The fourth subspecies, *etheridgei*, was described by Grismer (1990) as an insular form restricted to Isla Cerralvo off the coast of Southern Baja California in the Sea of Cortés.

Snout morphology and colour pattern are highly variable within *R. lecontei*, with the four subspecies

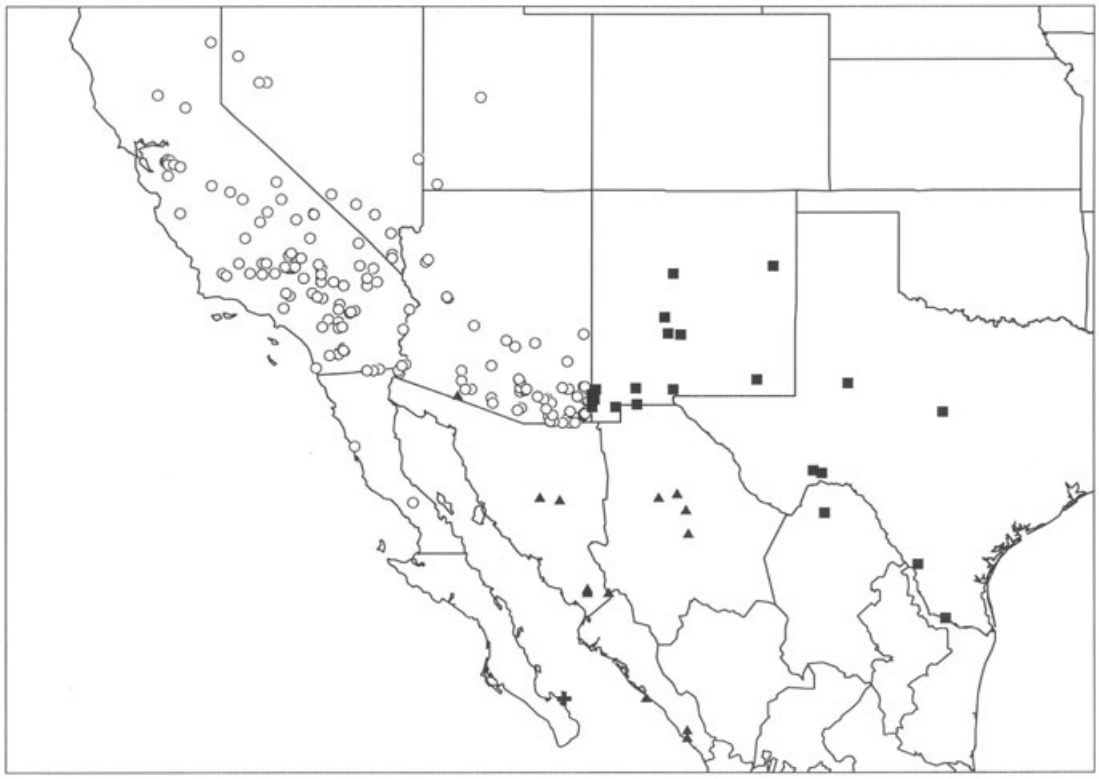

Figure 1. Map of collection localities of individuals analysed shown as circles (*Rhinocheilus lecontei lecontei*), squares (*R. l. tessellatus*), triangles (*R. l. antonii*) and crosses (*R. l. etheridgei*).

distinguished by colour pattern, morphology or scalation. The *antonii* subspecies is characterized by fewer (17 on average) and longer saddles that are spaced relatively close together (Klauber, 1941). The most recently described subspecies, *etheridgei*, features a square loreal scale, relatively short anterior temporal scales, shallow dorsal saddles (i.e. not contacting ventral scales) and large body size (Grismer, 1990). Distinguishing characteristics of *lecontei* are a large number of short dorsal saddles (25 on average), a high degree of lateral graininess and a relatively downturned snout (Klauber, 1941). Finally, *tessellatus* is characterized by a sharply upturned rostral scale, a colour pattern similar to *lecontei*, and a lower ventral scale count (200 on average for males and 195 on average for females; *lecontei* averages of 208 for males and 203 for females; Klauber, 1941). Individuals within all subspecies vary in colour pattern and ventral scale counts, and *tessellatus* also exhibited variation in degree of uplift of the rostral scale (Klauber, 1941). Statistics were limited to the calculation of mean, range, interquartile range and coefficient of variation for certain characters.

The goals of this study were to use univariate and multivariate statistical analyses to quantify the variation within and between subspecies of *R. lecontei* and to test the hypothesis that the subspecies are morphologically distinct. Canonical correlation analyses were also used to look for associations between morphological and environmental variables in order to discover the nature of the relationship between geographical patterns of climate and habitat and geographical patterns in morphology.

MATERIAL AND METHODS

I examined a total of 266 preserved specimens from throughout *R. lecontei*'s geographical distribution, representing all four subspecies (Fig. 1). Subspecies status was assigned based on collection locality and geographical ranges of subspecies as illustrated in Medica (1975) and Stebbins (1985). Morphological trait abbreviations are given in Table 1. Institutions from which specimens were obtained and catalogue numbers of specimens are given in the Appendix.

SCALATION

Seven scale counts were made: two from the body and five from the head (Table 1). Ventral scales (VENT)

Table 1. Descriptions of and codes used for scalation, colour pattern and linear morphometric (abbreviated to Lin morph here and in subsequent tables) variables

Variable	Code
Scalation	
Number of ventrals	VENT
Number of subcaudals	SUB
Number of preorbitals	PRE
Number of postorbitals	POST
Number of supralabials	SLAB
Number of infralabials	ILAB
Number of sublabials contacting first chin shield	SCS
Colour pattern	
Number of dark dorsal saddles	DDS
Number of dark caudal saddles	DCS
Dark dorsal saddles contacting ventral scales	SOV
Length of dark dorsal saddles	SAL
Graininess between dorsal saddles	GBS
Graininess within dorsal saddles	GWS
Degree of light coloration behind the jaw	LBJ
Degree of dark coloration behind the jaw	DBJ
Lateral blotches between saddles	LIB
Ventral blotches	VSB
Lin morph (in mm)	
Snout–vent length	SVL
Tail-length	TL
Head width	HW
Head length	HL
Snout width	SW
Snout length	SL

were counted according to Dowling's (1951) method, and the number of subcaudals (SUB), which are undivided in *R. lecontei*, began with the first scale directly posterior to the vent and did not include the terminal scale at the tail tip. All other scale counts were made on the left side of the head.

Colour pattern

Ten colour pattern variables were scored for each individual (Table 1). The number of dark dorsal saddles (DDS) was counted from the first whole saddle posterior to the head and ending with the last saddle entirely anterior to the vent. The number of dark caudal saddles (DCS) began with the first saddle at or posterior to the vent, and ended with the last saddle contacting caudal scales. Any blotches entirely on the terminal scale were not counted. SOV was determined by calculating the proportion of saddles that contacted the ventral scales on the right-hand side of each individual. SAL was obtained by calculating the average number of scales constituting the length of saddles at the following points along each individual: the first saddle, the saddle a third of the body length from the head, the saddle two thirds of the body length from the head, and the last saddle.

Graininess between (GBS) and within (GWS) saddles was calculated by averaging the number of scales alternately pigmented in the respective areas at the same points used to calculate SAL along the individual. Both the degree of light (LBJ) and dark (DBJ) coloration behind the jaw were assessed based on the level to which the coloration extended: (1) below the mouth, (2) as far as the mouth, (3) above the mouth but below the eye's midpoint, and (4) above the eye's midpoint (4). The presence of blotches (LIB) between the saddles formed by coalesced grains was described as: (1) little to no coalescence, (2) small disconnected blotches, or (3) a single large blotch. Finally, ventral blotches (VSB) were accounted for by calculating the percentage of ventral scales with one or more blotches. All lateral colour pattern characters were scored from the right-hand side of each individual.

Morphometrics

Two types of morphometric variable were used for this study, the first comprising linear distance measurements, the second derived from geometric morphometric analyses of landmark configurations (Bookstein, 1989, 1991) digitized from lateral- and dorsal-view images of a subset of 125 specimens. I measured six linear morphometric characters (Table 1). SVL and TL were measured using a meter stick, and all four head characters (HW, HL, SW, SL) were measured with Fowler Ultra-Cal III digital calipers to a hundredth of a millimetre.

I captured both lateral and dorsal images of the head using a Cohu Solid State video camera with a 55 mm Nikon Micro-NIKKOR lens and the software program NIH Image 1.61. Two configurations for each specimen were created to account for the limitations of a two-dimensional representation of a three-dimensional organism. The 22 landmarks per view used in this study represent points that are easily recognizable in all specimens examined, demarcated by head scales (Fig. 2). The software program TPSDIG was used to digitize landmarks on each image, and the landmark coordinates of individual configurations were compiled into files for comparison.

A tangent configuration was obtained by averaging the (x, y) coordinates of each individual configuration. It represents the point of tangency between the specimen's non-linear shape space and the approximating tangent space in which multivariate statistical analyses are performed (Rohlf, Loy & Corti, 1996). Each individual configuration is superimposed onto the tangent configuration and deformed such that the individual landmarks correspond to the tangent land-

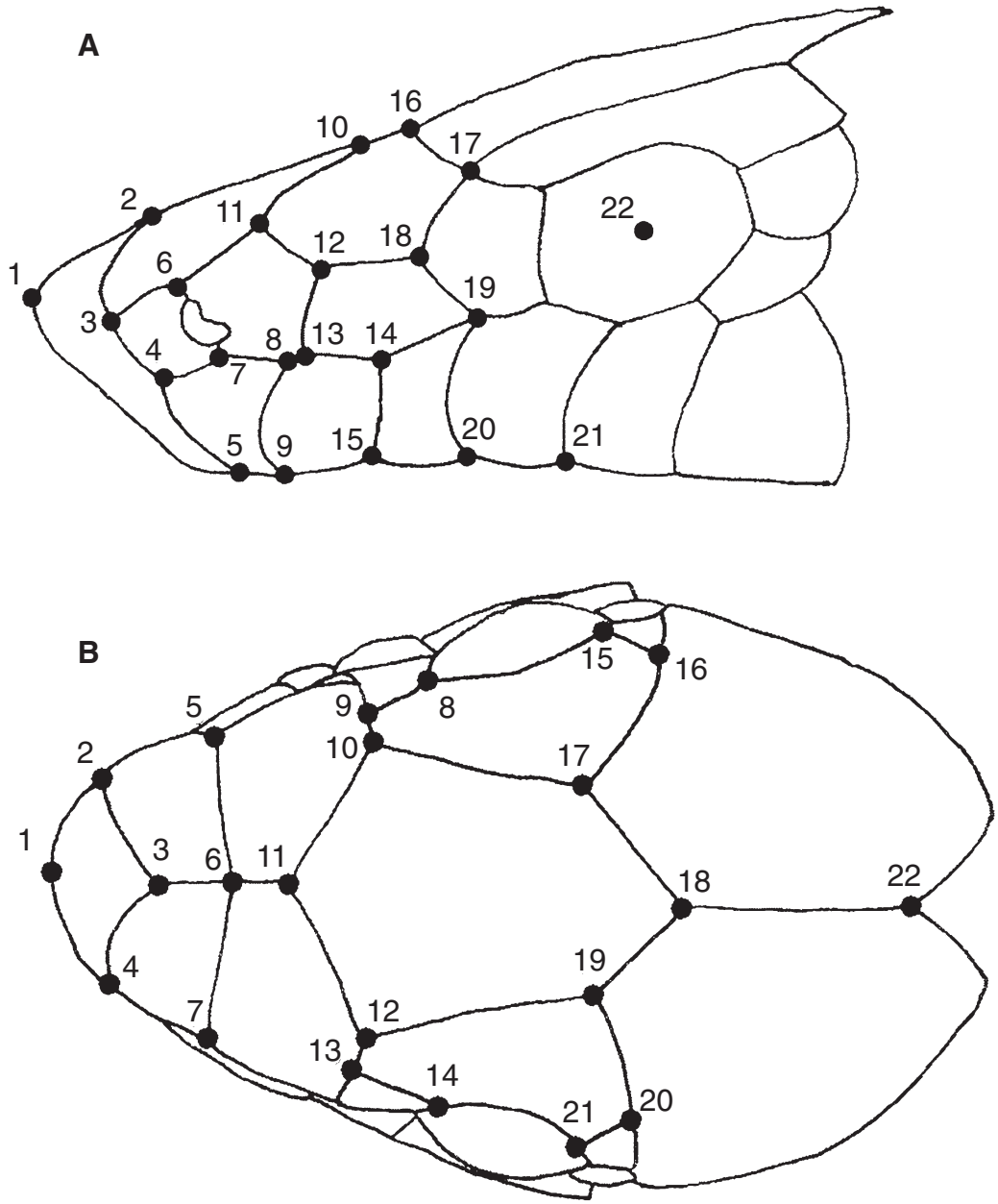

Figure 2. Landmarks used in geometric morphometric analysis. Numbers indicate order of digitization. A, top view. B, side view.

marks. The deformation is achieved by applying the thin-plate spline interpolating function (Bookstein, 1989), from which parameters can be obtained that correspond to partial-warp scores.

The columns of the weight matrix, W, represent these scores for each individual in the analysis, which are represented by the rows (Rohlf, 1993). W can also include the uniform component as the last two columns. These scores can be used as variables in multivariate statistical analyses that compare differences in shape among specimens (Corti & Crosetti, 1996; Rohlf *et al*., 1996). Here, a canonical discriminant analysis was applied to W (obtained using the program TpsRegr). Centroid sizes for each individual were obtained by calculating the square root of the sum of squared distances between each landmark and the centroid of the configuration (Bookstein, 1991). All thin-plate spline programs used in the analysis were written by F. J. Rohlf and can be obtained at life.bio.sunysb.edu/morph/soft-tps.html.

Table 2. Codes used to score environmental variables

Climate	Vegetation	Soil
1 = dry tropical desert	1 = desert scrub	1 = entisol
2 = dry subtropical desert	2 = semidesert scrub	2 = aridisol
3 = dry subtropical semidesert	3 = sclerophyllous vegetation	3 = mollisol
4 = highland	4 = semidesert scrub and woodland	4 = alfisol
5 = Mediterranean	5 = grassland	
	6 = cold needleleaf forest	
	7 = boreal forest	

Characterization of the environment

Environment was scored for each individual based on locality of collection in order to assess correlations between habitat and morphology. Climate, vegetation type, and soil type were determined using maps from Strahler & Strahler (1989). Classifications of climate were dry tropical desert, dry subtropical desert, dry subtropical semidesert, highland and Mediterranean. Vegetation types were desert scrub, semidesert scrub, sclerophyllous vegetation, semidesert scrub and woodland, grassland, cold needleleaf forest and boreal forest. Soil classifications were entisol, aridisol, mollisol and alfisol. For ecomorphological analyses, habitat types were assigned a numerical score based on increasing density of vegetation, increasing rainfall for climate, and decreasing particle size for soil (Table 2). In addition, latitude and longitude were obtained by identifying the specific collection locality for each specimen on detailed maps in the software program Street Atlas USA, v. 6.0 and at http://www.topozone.com.

Statistical analysis

The effect of scale was eliminated from the linear morphometric, scalation and colour pattern variables with a z-transformation that produced a mean of 0 and variance of 1. Sexual dimorphism was evaluated using general linear models (GLM; SAS, v. 8.1). Means were adjusted to body size (snout–vent length: SVL) for the colour pattern and linear morphometric datasets. Means of the geometric morphometric datasets were adjusted to centroid size (Bookstein, 1991). Differences in the regression coefficients (slopes) of the variables on SVL or centroid size for both sexes were assessed using the Type III Sum of Squares significance of the interaction between SVL and sex. For variables with equal slopes between the sexes, adjusted means were compared using a t-test. The number of scales does not vary ontogenetically in snakes, so adjusted means of scalation variables were compared directly using a t-test. Separate analyses were performed for each dataset, and subspecies were pooled.

Subspecies differentiation at the level of individual characters was assessed using general linear models (GLM; SAS, v. 8.1) similar to those used to investigate sexual dimorphism. Means were adjusted to SVL for colour pattern and LMV, and GMV were excluded from the analysis. Differences in the regression coefficients (slopes) of the variables on SVL for subspecies were assessed using the Type III Sum of Squares significance of the interaction between SVL and subspecies. For variables with equal slopes for all subspecies, adjusted means were compared using a t-test. Separate analyses were performed for each sex. Only one specimen of each sex was available for *etheridgei*, so that subspecies was excluded from the analysis.

Canonical discriminant analysis (CANDISC; SAS, v. 8.1) was used to test the null hypothesis that all subspecies are morphologically indistinguishable. CANDISC compares subspecies using a canonical correlation analysis that maximizes the correlation between a set of quantitative variables and a classification variable. The procedure produces canonical variables (CAN1, 2 and 3) that are linear combinations of the originals. The first canonical correlation (CC) has the highest multiple correlation with subspecies. Each successive CC identifies the orthogonal linear combination of the variables with the highest multiple CC with subspecies. The total number of CCs produced is determined by the number of original variables or the number of classes minus one, whichever is smaller. For each CC, CANDISC tests the null hypothesis that it and all following correlations are equal to zero. Separate analyses were carried out for each dataset (i.e. scalation, colour pattern, etc.). The geometric morphometric dataset for females was excluded due to insufficient sample size.

Canonical correlation analysis (CANCORR; SAS, v. 8.1) was used to investigate the relationship between morphological and environmental variables. This procedure analyses the correlation between two sets of variables. The scalation, colour pattern and LMV were analysed as a single dataset. The geometric morphometric datasets were excluded from the analysis,

because the partial warp variables have no biological significance in and of themselves (Rohlf, 1998).

RESULTS

Sample size, mean and standard deviation for raw scalation, colour pattern and LMV are reported by sex and subspecies in Table 3. SO was removed from further analyses, since every individual had the same value.

Sexual dimorphism

Sexual dimorphism was found in all datasets (Table 4). Three out of seven scalation variables were sexually dimorphic. Males had more ventrals, more subcaudals, and fewer infralabials than females. Two of the colour pattern characters (graininess between saddles and ventral blotching) were sexually dimorphic in their regression slopes on SVL. Of the remaining eight characters, number of caudal saddles and dark coloration behind the jaw differed for males and females. The linear morphometric characters showed the highest degree of sexual dimorphism. Snout width did not regress equally on SVL for males and females, and males had longer tails and shorter heads and snouts. Only head width did not differ between the sexes.

Of the 40 side-view partial warps, the regression coefficients of three variables on centroid size were significantly different between the sexes. Two of the remaining partial warps showed statistically significant sexual dimorphism. For the top view, one partial warp did not vary with centroid size equally for both sexes. Of the remaining variables, three showed significant sexual dimorphism. Because the individual partial warp scores have no relevant biological significance (Rohlf, 1998), this analysis was used only to determine if the sexes should be treated separately.

Based on the relative proportion of unequal slopes and adjusted means, head shape as determined by geometric morphometrics was the least sexually dimorphic, while linear morphometric characters were the most sexually dimorphic. Almost half of the scalation characters were sexually dimorphic, and there was little sexual dimorphism in colour pattern. For all datasets, males and females were analysed separately for the remaining statistical tests with the exception of the geometric morphometric dataset, for which only males were analysed due to low female sample size ($N_m = 89$).

Differentiation of subspecies

An analysis of covariance on subspecies showed more differentiation among males than females (Table 5). Among males, colour pattern characters separated *antonii* from both *lecontei* and *tessellatus*, and linear morphometric characters differentiated *lecontei* from *tessellatus*. Only snout length was significantly divergent for all three subspecies. Among females, there were few strong patterns of subspecies differentiation, but *antonii* and *lecontei* were most distinct. Scalation variables were generally poor at separating subspecies for either sex.

For the canonical discriminant analysis of scalation variables, only the first CC with subspecies was significant for both males and females (CC1 = 0.490, $P < 0.005$ and CC1 = 0.650, $P < 0.006$, respectively). The statistical significance of a CC is the probability that it and all subsequent correlations are zero, and the *P*-value of the first CC is equal to that of the Wilks' lambda, a multivariate test of the equality of group means. The first eigenvalue, the ratio of between-class variance to within-class variance, was low: 0.316 with a proportion of 0.737 for males, and 0.732 with a proportion of 0.622 for females. The canonical coefficients indicate that ventrals, subcaudals, postorbitals and sublabials contribute the most to CAN1 for the males. Ventrals, subcaudals and preorbitals are most influential for females (Table 6). For the males, only the *antonii–tessellatus* ($P < 0.0124$) and *lecontei–tessellatus* ($P < 0.0002$) Mahalanobis distances were significant, while *antonii–etheridgei* ($P < 0.0118$), *antonii–lecontei* ($P < 0.0048$) and *etheridgei–lecontei* ($P < 0.0277$) were significant for females (Table 7). A frequency histogram of CAN1 showed little separation of subspecies for either sex (Fig. 3).

As for the scalation characters, only the first CC for the colour pattern characters was significant for both males and females (CC1 = 0.644, $P < 0.0001$ and CC1 = 0.681, $P < 0.0233$, respectively). Its eigenvalue was 0.709 with a proportion of 0.735 for males and 0.866 with a proportion of 0.610 for females. For males, CAN1 was largely composed of the sum of saddle length and lateral blotches; for females, number of dorsal and caudal saddles, saddle length, graininess within saddles, lateral blotches and ventral blotches were all strong contributors to CAN1 (Table 6).

The pairwise Mahalanobis distances between *antonii–lecontei* ($P < 0.0001$), *antonii–tessellatus* ($P < 0.0005$) and *lecontei–tessellatus* ($P < 0.0268$) were significant for males, but only *antonii–lecontei* was significant for females ($P < 0.0050$; Table 7). A frequency histogram of CAN1 for the males showed the general overlap of all subspecies (Fig. 4A). The histogram for the females showed a differentiation of *antonii* from *lecontei* and *etheridgei*, with *tessellatus* spanning them all (Fig. 4B). In general, colour pattern was more able to differentiate among the subspecies in females than males, even though colour pattern characters did not show a high degree of sexual dimorphism.

Table 3. Sample sizes (*N*), means and standard deviations (SD) of unstandardized scalation, colour pattern and linear morphometric variables by subspecies and sex

	antonii (*N* = 20)						*etheridgei* (*N* = 2)				*lecontei* (*N* = 216)						*tessellatus* (*N* = 28)					
	Males (*N* = 14)			Females (*N* = 6)			Males		Females		Males (*N* = 143)			Females (*N* = 73)			Males (*N* = 20)			Females (*N* = 8)		
	N	Mean	SD	*N*	Mean	SD	*N*	Mean	*N*	Mean	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD
Scalation																						
VENT	14	198.36	4.60	6	191.00	4.90	1	205.00	1	204.00	126	202.58	7.55	57	200.12	7.11	8	197.00	4.07	2	190.50	9.19
SUB	14	49.43	2.17	6	49.33	10.86	1	52.00	1	48.00	117	49.75	2.54	55	43.82	3.13	7	50.43	5.65	2	48.00	1.41
PRE	14	1.21	0.43	6	1.00	0.00	1	1.00	1	1.00	106	1.15	0.36	45	1.13	0.34	7	1.14	0.38	2	1.00	0.00
POST	14	2.00	0.00	5	2.00	0.00	1	2.00	1	2.00	106	2.01	0.10	45	2.04	0.21	8	2.25	0.46	2	2.00	0.00
SLAB	14	8.07	0.27	5	8.2	0.45	1	8.00	1	8.00	104	8.05	0.35	45	8.11	0.32	8	8.13	0.35	2	8.00	0.00
ILAB	14	8.00	0.39	6	8.17	0.75	1	9.00	1	10.00	103	8.06	0.48	45	8.20	0.55	6	7.67	0.52	2	8.00	0.00
SCS	14	3.64	0.50	5	3.80	0.45	1	3.00	1	3.00	104	3.57	0.52	45	3.67	0.56	7	3.57	0.53	2	3.50	0.71
Colour pattern																						
DDS	14	18.50	6.32	6	21.33	4.37	1	26.00	1	26.00	143	26.41	4.85	73	24.96	4.79	20	26.40	4.13	8	26.00	2.51
DCS	14	7.00	1.84	6	7.17	1.72	1	10.00	1	10.00	135	9.24	1.67	71	8.15	1.42	19	9.37	1.71	8	8.88	1.46
SOV	14	79.99	32.73	6	51.52	45.35	1	3.80	1	0.00	143	38.93	31.55	73	49.37	34.18	20	34.95	33.29	8	24.61	30.02
SAL	14	9.57	2.66	6	7.79	2.84	1	6.00	1	6.00	105	6.42	1.36	45	6.72	1.46	8	6.13	0.74	2	5.38	0.88
GBS	14	4.32	3.89	6	5.04	4.07	1	12.50	1	18.25	105	6.19	5.31	45	6.92	5.84	8	8.92	5.19	2	3.75	4.24
GWS	14	19.25	10.01	6	8.08	6.38	1	19.00	1	18.00	105	15.19	5.07	45	16.72	6.30	8	15.72	5.93	2	14.63	5.13
LBJ	14	0.86	0.53	6	0.83	0.41	1	1.00	1	1.00	143	1.67	1.02	73	1.56	0.93	20	1.15	0.49	8	1.00	0.00
DBJ	14	1.14	0.36	6	1.17	0.41	1	1.00	1	1.00	143	1.34	0.66	73	1.18	0.45	20	1.15	0.37	8	1.13	0.35
LIB	14	1.57	0.94	6	2.00	1.10	1	2.00	1	3.00	142	1.75	0.75	73	1.90	0.84	20	2.40	0.82	8	2.75	0.46
VSB	14	19.40	19.22	6	49.03	13.82	1	4.40	1	9.30	105	4.71	9.79	44	14.68	21.36	8	13.25	23.87	2	40.15	43.20
Lin morph																						
SVL	14	671.50	95.38	6	498.33	137.23	1	1021.00	1	851.00	143	581.53	110.38	73	545.19	89.65	20	565.55	84.18	8	513.13	80.53
TL	14	104.86	12.43	6	78.33	16.27	1	143.00	1	115.00	134	91.27	16.05	71	77.58	11.69	19	95.05	13.91	8	84.38	14.94
HW	14	11.71	0.99	5	10.03	2.87	1	17.21	1	14.28	114	10.99	1.54	57	10.49	1.21	15	10.22	0.99	7	9.64	1.40
HL	14	16.88	1.60	6	14.83	1.98	1	22.02	1	19.74	119	16.08	1.81	60	15.89	1.57	20	15.40	1.32	8	15.65	1.72
SW	14	3.62	0.31	6	3.22	0.78	1	3.47	1	3.50	122	3.29	0.43	60	3.32	0.46	20	2.91	0.42	8	3.14	0.44
SL	14	2.43	0.38	6	2.07	0.53	1	3.38	1	2.25	122	2.03	0.39	60	2.08	0.37	20	1.85	0.26	8	1.98	0.54

Table 4. Tests for sexual dimorphism in standardized scalation, colour pattern and linear morphometric characters. Linear morphometric and colour pattern means adjusted to SVL

	N	=P > *F* for equal slopes	Adjusted mean		P > |*T*| for equal means
			males	females	
Scalation					
VENT	215	–	0.1165	–0.2629	0.01
SUB	203	–	0.3949	–0.8577	0.0001
PRE	182	–	0.0382	–0.0905	0.4294
POST	182	–	–0.0257	0.0626	0.5896
SLAB	180	–	–0.0511	0.1224	0.2899
ILAB	178	–	–0.1045	0.0889	0.0343
SCS	179	–	–0.0502	0.1193	0.3019
Colour pattern					
DDS	266	0.4554	0.0717	–0.1150	0.1682
DCS	255	0.3539	0. 1958	–0.3479	0.0001
SOV	266	0.3782	–0.0681	0.1024	0.2070
SAL	182	0.2357	–0.0285	0.0178	0.7798
GBS	182	0.0499			
GWS	182	0.0611	–0.0202	0.1243	0.3677
LBJ	266	0.7239	0.0413	–0.0976	0.3057
DBJ	266	0.5686	0.0841	–0.1929	0.0407
LIB	265	0.1529	–0.0608	0.1790	0.0757
VSB	181	0.0013			
Lin morph					
TL	254	0.0982	0.1655	0.0318	0.0001
HW	214	0.1105	0.0169	0.0060	0.8973
HL	229	0.2630	–0.0744	0.1711	0.0001
SW	232	0.0207			
SL	232	0.2317	–0.0846	0.2180	0.011

For the linear morphometric characters, all three CCs were significantly different from zero for males (CC1 = 0.555, $P < 0.0001$; CC2 = 0.440, $P < 0.0001$; CC3 = 0.337, $P < 0.0037$). Their eigenvalues were 0.446, 0.240 and 0.128, with proportions of 0.547, 0.295 and 0.158, respectively. Only the first CC was significant for females (CC1 = 0.645, $P < 0.0001$), and its eigenvalue was 0.712 with a proportion of 0.709. CAN1 for males was largely a difference between SVL and snout width, though head length and snout length were also influential. Snout width and snout length were primarily responsible for CAN2, and all linear morphometric variables except snout length were important in CAN3. The largest coefficients of CAN1 for females belonged to SVL, tail length, head width and snout width (Table 6).

All pairwise Mahalanobis distances were highly significant for males. The *antonii–etheridgei* ($P < 0.0001$), *etheridgei–lecontei* ($P < 0.0002$), *etheridgei–tessellatus* ($P < 0.0001$) and *lecontei–tessellatus* ($P < 0.0139$) subspecies pairs were significant for females (Table 7). CAN1 for males primarily separated *etheridgei* from the other subspecies, while *antonii* and *etheridgei* were most differentiated from *tessellatus* along CAN2, all of which were overlapped by *lecontei* (Fig. 5A). There was no clear segregation along CAN3 (Fig. 5B). CAN1 for females, as for males, primarily separated *etheridgei* from the remaining subspecies (Fig. 6). The linear morphometric characters are indicative of size, and the largest contributor to CAN1 for both sexes was SVL. It is therefore not surprising that CAN1 separated *etheridgei*, the largest subspecies, from the others. CAN2 separated *tessellatus* from the remaining subspecies, primarily due to snout width, snout length and tail length.

None of the CCs for the geometric morphometrics (only males were analysed) were statistically significant (CC1 = 0.825, $P < 0.0873$ for side view; CC1 = 0.781, $P < 0.0715$ for top view). All Mahalanobis distances for both views are non-significant, except between *lecontei* and *tessellatus*, which have the most divergent snout morphology (Klauber, 1941; $P < 0.0298$ for side view, $P < 0.0459$ for top view). The relative contributions of the partial warps to the canonical variables are not reported because of their lack of biological significance (Rohlf, 1998).

Table 5. Tests of subspecies differentiation for standardized scalation, colour pattern and linear morphometric characters. Linear morphometric and colour pattern means adjusted to SVL. $P > |T|$ for equal means; $-P > F$ for equal slopes non-significant, NS, non-significant; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$

	males			females		
	ant-lec	ant-tess	lec-tess	ant-lec	ant-tess	lec-tess
Scalation						
VENT	NS	NS	*	*	NS	NS
SUB	NS	NS	NS	*	NS	NS
PRE	NS	NS	NS	NS	NS	NS
POST	NS	NS	*	NS	NS	NS
SLAB	NS	NS	NS	NS	NS	NS
ILAB	NS	NS	*	NS	NS	NS
SCS	NS	NS	NS	NS	NS	NS
Colour pattern						
DDS	****	****	NS	*	*	NS
DCS	***	***	NS	–	–	–
SOV	***	***	NS	NS	NS	NS
SAL	****	****	NS			
GBS	NS	*	*	NS	NS	NS
GWS	–	–	–	*	NS	NS
LBJ	*	NS	*	NS	NS	NS
DBJ	NS	NS	NS	NS	NS	NS
LIB	NS	*	***	–	–	–
VSB	***	NS	*	**	NS	NS
Lin morph						
TL	NS	NS	**	*	NS	****
HW	–	–	–	NS	NS	NS
HL	NS	NS	*	NS	*	*
SW	NS	***	****	NS	NS	NS
SL	*	**	*	NS	NS	NS

Morphology–environment correlation

The canonical correlation analysis of the morphological variables (scalation, colour pattern and linear morphometrics combined) with the environmental variables revealed significant associations for both sexes. For the males, the first two CCs were significant (CC1 = 0.830, $P < 0.0001$; CC2 = 0.729, $P < 0.006$). Their eigenvalues were 2.217 and 1.134, with proportions of 0.554 and 0.284, respectively. Based on the standardized canonical coefficients of the variables, CAN1 is largely composed of light coloration behind the jaw and climate. Number of ventrals was the only scalation variable to be even moderately influential, and none of the linear morphometric variables had high loadings (Table 8). No morphological variables were strongly weighted in CAN2. Only ventrals, sublabials, number of dorsal saddles and dark coloration behind the jaw were moderately influential. All environmental variables except longitude had strong contributions to CAN2. I am reporting standardized canonical coefficients here instead of raw, because the environmental variables were not standardized.

For the females, only the first CC was significantly different from zero (CC1 = 0.953, $P < 0.0002$), but the second is reported as well, because it is only slightly insignificant (CC2 = 0.908, $P < 0.071$). Their eigenvalues were 9.826 and 4.723 with proportions of 0.565 and 0.272, respectively. Colour pattern and LMV along with longitude and vegetation contributed most to CAN1. Specifically, saddle length, graininess between saddles, light coloration behind jaw, tail length and head length were most influential (Table 8). None of the scalation variables weighed heavily on CAN1. Scalation variables were again largely unimportant in CAN2, with number of caudal saddles, saddle length, SVL and head length weighing most heavily. All environmental variables except soil were influential. Overall, climate was the most important environmental variable for males, and vegetation and longitude were most important for females. Longitude was least important for males, while soil was least important for females. Of the morphological variables, colour pattern was influential for both sexes, but linear morphometrics

Table 6. Raw canonical coefficients from canonical discriminant analysis of standardized scalation, colour pattern and linear morphometric variables for males and females. Coefficients are shown only for those canonical correlations that differed significantly from zero. Coefficients referred to in text are in bold

	Males			Females
Canonical correlation:	1	2	3	1
Scalation				
VENT	**–0.6432**			**0.9775**
SUB	**1.1505**	–	–	**–0.4120**
PRE	0.0425	–	–	**0.4222**
POST	**0.7470**	–	–	0.0636
SLAB	0.1445	–	–	–0.2806
ILAB	**–0.6233**	–	–	0.2323
SCS	0.1560	–	–	–0.2863
Colour pattern				
DDS	0.0998	–	–	**–0.7603**
DCS	–0.1072	–	–	**0.6476**
SOV	–0.0788	–	–	0.2274
SAL	**1.7281**	–	–	**–0.8803**
GBS	0.2260	–	–	0.3094
GWS	–0.3285	–	–	**0.6604**
LBJ	–0.1208	–	–	0.3973
DBJ	0.1430	–	–	–0.0385
LIB	**0.7059**	–	–	**–0.6844**
VSB	0.0089	–	–	**–0.4971**
Lin morph				
SVL	**1.5055**	0.2912	**0.6341**	**–2.6311**
TL	0.1477	–0.0182	**1.1288**	**1.1690**
HW	0.2352	0.3881	**–1.0922**	**–0.9257**
HL	**–0.4421**	–0.3095	**–1.3649**	0.2659
SW	**–1.3687**	**0.9893**	**0.6181**	**1.2749**
SL	**0.5014**	**0.4785**	0.3758	0.3694

was unimportant for males, while scalation was unimportant for females (Table 8).

Of the morphological and environmental variables that contributed heavily to CAN1 or 2 for males, number of dark dorsal saddles and light coloration behind the jaw were associated with vegetation and, to a lesser extent, climate and latitude (Fig. 7A). Dark coloration behind the jaw was associated with soil characteristics, and number of ventrals and sublabials were not closely associated with any influential environmental variable. For females, SVL and head length were positively associated with vegetation but negatively associated with longitude (Fig. 7B). Saddle length and tail length were negatively associated with climate, while graininess between saddles and the number of sublabials contacting the chin shields were positively associated with climate. Finally, light coloration behind the jaw was positively associated with latitude and negatively associated with longitude.

Clinal variation in a number of scalation and colour pattern variables was detected across both latitude and longitude. For males, number of dorsal and caudal saddles and light coloration behind the jaw were significantly and positively correlated with latitude, while saddle length, ventral blotches and tail length were negatively correlated with latitude. For females, number of ventrals, number of dorsal and caudal saddles, graininess within saddles and light coloration behind the jaw increased with latitude, while number of subcaudals, saddle length and ventral blotches decreased with latitude. Number of subcaudals, lateral blotches between saddles, ventral blotches and tail length increased with longitude, while number of ventrals, number of saddles, graininess within saddles, light coloration behind jaw and snout width decreased with longitude.

Clinal variation was discordant in females for several closely related variables. Number of ventrals increased with latitude, and number of subcaudals decreased ($r = 0.316$ and -0.312, respectively). Meanwhile, number of ventrals decreased with longitude, while number of subcaudals increased ($r = -0.521$ and 0.362, respectively). Also, tail length increased with longitude, while snout width decreased ($r = 0.239$ and -0.242, respectively). In both sexes, the number of dorsal saddles increased with latitude, while saddle length decreased. However, I do not consider this discordant variation, because it is biologically unfeasible to maintain the same number of dorsal saddles and make them longer without increasing the total length of the snake. For both sexes, colour pattern characters most often had significant correlations with one or more environmental variables, while linear morphometric characters were least associated with environment. In particular, number of dorsal saddles and light coloration behind the jaw were most frequently and most significantly associated with environmental variables. Climate was most frequently correlated with morphology, while longitude had no significant correlations with morphology.

DISCUSSION

Reports of sexual dimorphism in snakes are uncommon, and natural history differences between the sexes are largely unknown. A few studies have found that the sexes can differ in overall size, tail length, number of ventrals and number of subcaudals (Grobman, 1941; Dowling, 1950), but sexual differences in other characters such as colour pattern are rarely reported. Nevertheless, subtle sexual dimorphism can be detected in a wide range of characters when sample sizes are large (Arnold & Phillips, 1999). In

Table 7. Squared Mahalanobis distances between subspecies pairs and Wilks' lambda (WL) from each canonical discriminant analysis. Geo morph, geometric morphometric; NS, non-significant; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$

	Males			Females		
	etheridgei	*lecontei*	*tessellatus*	*etheridgei*	*lecontei*	*tessellatus*
Scalation						
antonii	8.10699 NS	0.70083 NS	4.79542 *	29.10938 *	6.28984 **	0.85328 NS
etheridgei		6.27103 NS	15.32116 NS	21.01334	23.72175 *	 NS
lecontei			5.88146 ***			4.23485 NS
	WL = 0.6806; $P < 0.0051$			WL = 0.3979; $P < 0.0058$		
Colour pattern						
antonii	14.03134 NS	6.59335 ****	8.11311 ***	17.38843 NS	7.43543 **	12.50043 NS
etheridgei		6.87966 NS	9.36127 NS		8.99279 NS	21.99851 NS
lecontei			3.57573 *			10.15257 NS
	WL = 0.4616; $P < 0.0001$			WL = 0.3308; $P < 0.0233$		
Lin morph						
antonii	46.18744 ****	1.9646 ***	4.11873 ***	48.77516 ****	2.16609 NS	1.88429 NS
etheridgei		52.57319 ****	49.00037 ****		34.53704 ***	43.40705 ****
lecontei			2.38069 ****			3.10645 *
	WL 0.4942; $P < 0.0001$			WL = 0.4488; $P < 0.0001$		
	Side view			Top view		
	etheridgei	*lecontei*	*tessellatus*	*etheridgei*	*lecontei*	*tessellatus*
Geo morph						
antonii	62.81816 NS	18.37954 NS	13.16866 NS	89.22945 NS	12.65088 NS	15.96535 NS
etheridgei		75.18506 NS	76.67361 NS		96.98019 NS	102.74939 NS
lecontei			12.06173 *			11.34806 *
	WL = 0.1086; $P < 0.0873$			WL = 0.1054; $P < 0.0715$		

R. lecontei, I found differences between the sexes in each dataset, with the highest incidence of sexual dimorphism among the linear morphometric variables. Such differences in *R. lecontei* may result from a number of factors, some of which may be operating simultaneously.

Sexual selection or ecological differences may cause the sexes to experience divergent selection pressures that result in dissimilar morphologies. The prevalence of sexual dimorphism among the LMV makes sense in light of the tendency for male *Rhinocheilus* to be larger than females. The trend for larger males is unusual in colubrid snakes and often indicates a mating system with male–male competition (Shine, 1978, 1994) or female oviparity (Fitch, 1981), both of which are found in *Rhinocheilus* (Carpenter, 1986). Intersexual differences in body size should therefore explain the differences observed in tail length, head length and snout length due to genetic correlation (Lande, 1980). This conclusion is consistent for tail length, strengthened by the fact that male snakes store their hemipenes in their tails, which consequently tend to be larger relative to SVL than in females. Many intersexual differences in linear morphometrics can

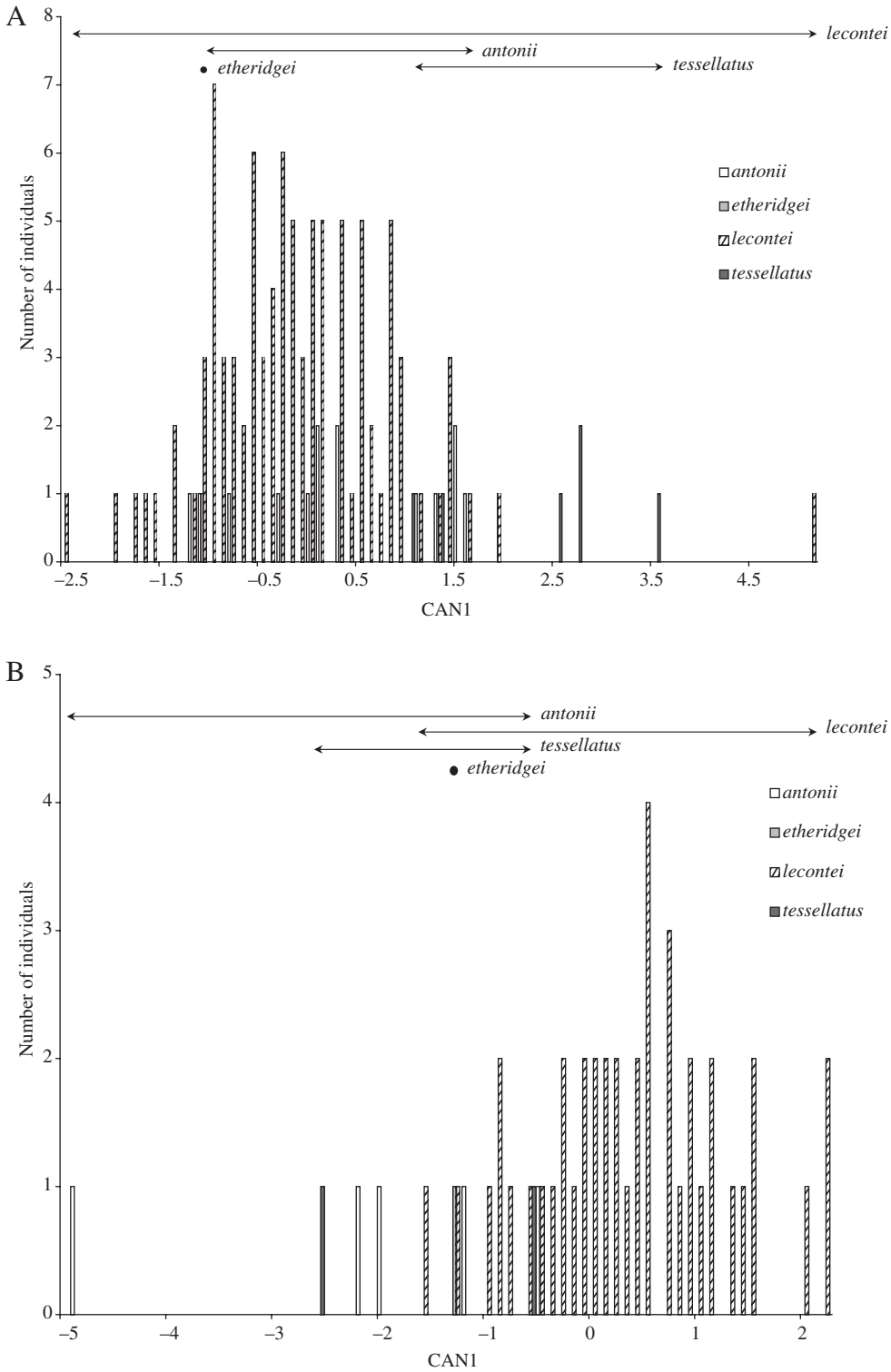

Figure 3. Frequency histograms of CAN1 for scalation characters: (A) males and (B) females. The subspecies *antonii* is indicated in white, *etheridgei* in light grey, *lecontei* with diagonal lines and *tessellatus* in dark grey. Subspecific spread across the histogram is indicated by labelled range arrows above each graph.

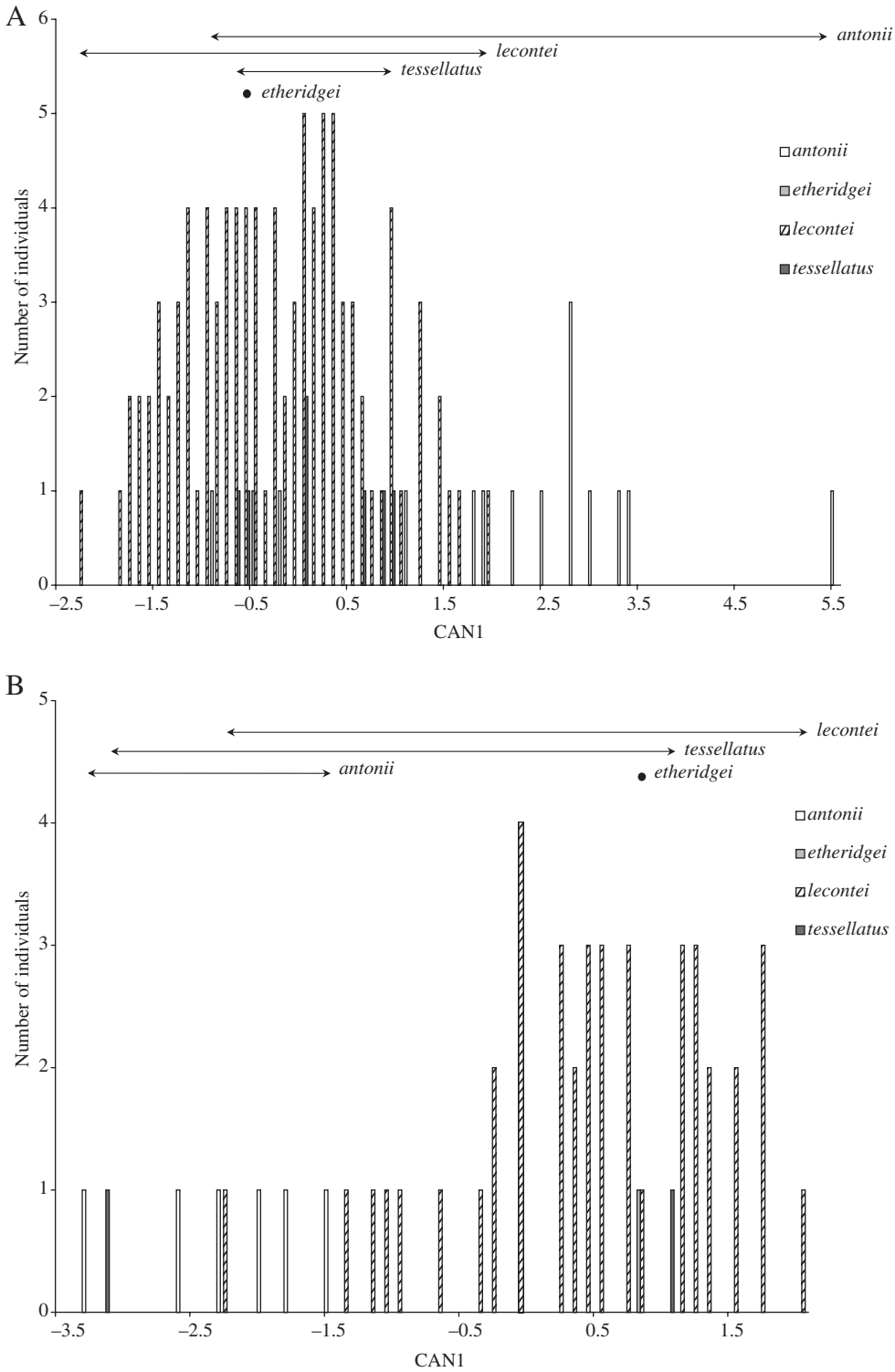

Figure 4. Histograms of CAN1 for colour pattern characters: (A) males and (B) females.

therefore be explained by sexual selection favouring larger males.

Head size, on the other hand, is correlated with maximum size of ingestible prey (e.g. Savitsky, 1983) and has little influence on mating behaviour (Carpenter & Ferguson, 1977; Shine *et al.*, 1981; Charles, Field & Shine, 1985). Females are smaller in body size, but they have relatively longer heads and snouts, a

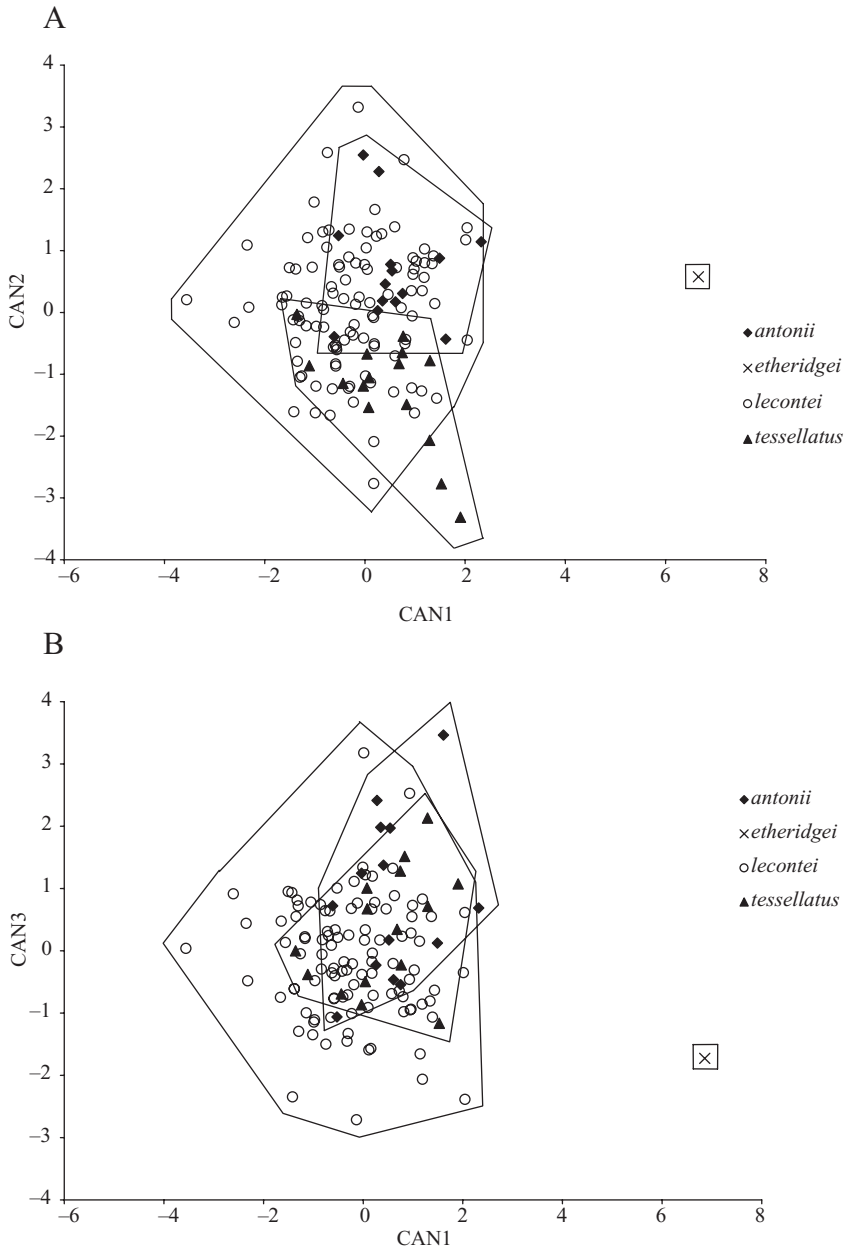

Figure 5. For linear morphometric characters: scatterplots of (A) CAN1 on CAN2 and (B) CAN1 on CAN3 for males.

sexually dimorphic trait that may have evolved via dietary divergence instead of sexual selection (Shine, 1989, 1991). Diet studies in *Rhinocheilus* have only investigated variation in diet and did not examine sexual divergence in prey type (Rodriguez-Robles & Greene, 1999; Rodriguez-Robles, Bell & Greene, 1999). If longer heads in females are a result of intersexual dietary divergence, I would expect females to consume larger prey items overall, such as mammals. Sexual dimorphism in snout length may result from a genetic correlation with head length, ecological differences between the sexes or a combination of both. I found a pronounced correlation between head length and snout length ($r^2 = 0.327, P < 0.0001$) that may indicate a purely pleiotropic explanation for sexual divergence in snout length. Alternatively, the longer rostral scale in females may have selective advantages, perhaps related to diet or nest excavation.

I also found that the sexes differed in the degree of subspecies separation for two of the three datasets for which an intersexual comparison was made. One possible explanation for this result is sex-biased dis-

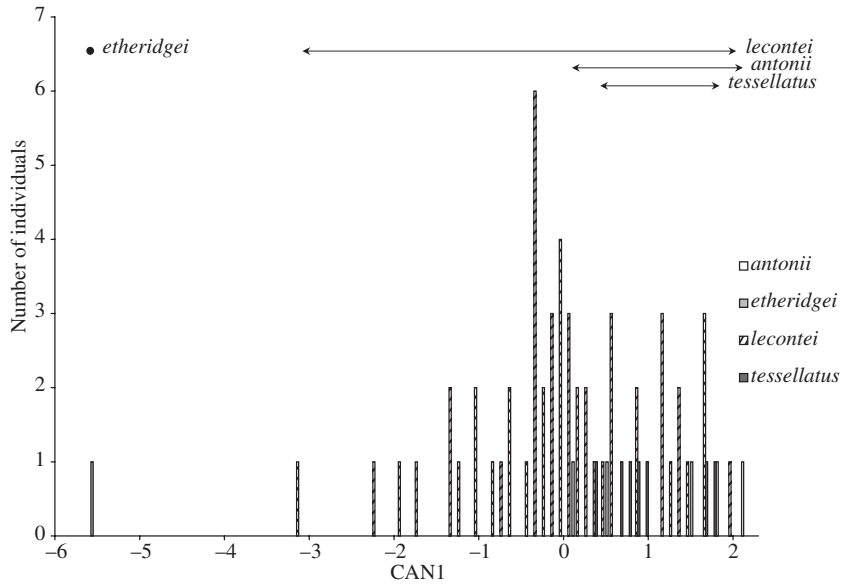

Figure 6. Histogram of CAN1 for linear morphometric characters for females.

persal, such that gene flow is dominated by female movement, resulting in more pronounced subspecies differentiation among males than females. If males have greater subspecies differences because of lower levels of gene flow among populations, one would expect to find a higher percentage of females dispersing in the field. As such, females would be encountered more often by collectors and would therefore be more prevalent in museum collections. Based on the sex ratio of the museum specimens available for this study ($N_m = 178$, $N_f = 88$), however, males are actually encountered more often. Thus, sex-biased dispersal does not seem to explain the observed dimorphism.

Two morphometric methods were used in this study: traditional linear morphometrics and geometric morphometrics. Linear morphometrics involves linear measurements of lengths and distances, whereas geometric morphometrics are used to evaluate variation in shape. Because the linear morphometrics included tail length and SVL, the two datasets cannot be compared directly. However, several informal conclusions can be drawn. The two types of morphometrics are not equal in their ability to detect either geographic variation or sexual dimorphism. Geographic variation was most apparent in the linear morphometric characters but was barely detectable in the geometric morphometric characters. Furthermore, the linear morphometric characters exhibited the highest degree of sexual dimorphism, while the geometric morphometric characters had the lowest. The simplest interpretation of these results is that the subspecies differ in size but not shape. Even so, much of the size differentiation can be accounted for by the much larger size of the island subspecies, *etheridgei*.

Strong associations of morphology with environment were most apparent for colour pattern. In particular, number of dorsal and caudal saddles, saddle length and light coloration behind the jaw were important in both univariate and multivariate correlation analyses for both sexes. Variation in saddle characteristics is most readily observed in museum collections. Populations belonging to *R. l. antonii* frequently have much longer and fewer saddles than those for the other three subspecies, a trend observed in both sexes. In addition, light coloration behind the jaw appears to be present only in specimens from the Central Valley of California, which claims its own climate (Mediterranean), vegetation (grassland) and soil (entisol). Such a distinct geographical pattern of coloration on the head may be the result of limited gene flow out of the Central Valley, rather than selection actively maintaining that colour morph. An analysis using molecular markers could determine the genetic structure and levels of gene flow among populations in that region.

Variation in morphology was the focus of this study, because the original subspecies descriptions used morphological traits, and a molecular survey of this widespread species was beyond the scope of this study. However, molecular methods are becoming increasingly useful in elucidating subspecies relationships in snakes. In particular, two recent studies have uncovered intraspecific phylogenetic relationships that disagree with the recognized subspecies. Burbrink,

Table 8. Standardized canonical coefficients from canonical correlation analysis of environmental variables with morphological variables (scalation, colour pattern and linear morphometric). All canonical correlations shown, except 2nd of females, differ significantly from zero. Wilks' lambda for each analysis is also shown. Coefficients mentioned in text are in bold. *** $P < 0.001$, **** $P < 0.0001$

	Males		Females	
Canonical correlation:	1	2	1	2
Scalation				
VENT	–0.4562	**0.4574**	–0. 1790	0.1049
SUB	–0.0142	0.0458	–0.4267	–0.2799
PRE	0.0842	–0.2174	–0.0286	–0.0237
POST	–0.0128	–0.0138	0.2257	0.3229
SLAB	0.0156	0.0951	0.1662	–0.1413
ILAB	–0.1502	**0.4331**	–0.1543	0.1047
SCS	0.0352	–0.2292	0.2311	0.2751
Colour pattern				
DDS	0.3167	**0.4465**	0.0196	0.1493
DCS	–0.0257	–0.3326	0.4129	**0.6162**
SOV	0.2470	–0.0128	–0.3955	–0.3947
SAL	0.0260	–0.3296	**0.5116**	**1.2849**
GBS	–0.1837	–0.0666	**–0.6501**	0.2337
GWS	–0.1837	0.1242	–0.2621	–0.1468
LBJ	**0.7271**	0.1632	**–0.5347**	–0.2206
DBJ	–0.0958	**–0.4367**	–0.0008	0.2637
LIB	0.1420	0.0798	0.4841	–0.3132
VSB	–0.1521	0.0899	0.2600	–0.0731
Lin morph				
SVL	–0.2034	–0.2958	–0.2370	**0.6452**
TL	0.0938	–0.1941	**1.1229**	0.1951
HW	–0.0304	–0.0608	0.1156	0.3459
HL	0.0341	0.1894	**–0.6739**	**–1.1929**
SW	0.1852	0.1643	–0.4111	–0.3447
SL	–0.0786	–0.0568	0.3938	0.306
Environmental				
LAT	0.1272	**0.8129**	–0.1435	**–1.0759**
LONG	0.0444	0.2695	**0.8809**	**–0.8406**
CLIM	**1.2247**	**–0.9151**	–0.0471	**–0.7106**
VEG	–0.3846	**0.6926**	**0.5838**	**1.3866**
SOIL	0.0024	**–0.5092**	0.4063	0.4547
Wilks' lambda	0.081****		0.003***	

Lawson & Slowinski (2000) provide evidence that subspecies designations based on a small number of colour pattern characters may grossly misrepresent phylogenetic relationships, as determined by mitochondrial genetic markers. In addition, Bronikowski & Arnold (2001) conducted a species-wide survey of the mitochondrial cytochrome *b* gene and found geographically related clades that were paraphyletic with respect to subspecies. Additional information about genetic differentiation among populations of *R. lecontei* will undoubtedly shed more light on its subspecific structure.

Few of the original characters used to distinguish *R. lecontei* subspecies (Klauber, 1941; Grismer, 1990) were found to be truly diagnostic, and only about half of them were supported by the analysis of covariance for separation of subspecies (Table 5). The *antonii* subspecies did indeed have significantly fewer and longer dorsal saddles for both males and females, but neither character was diagnostic.

Grismer (1990) described three diagnostic characters for *etheridgei* along with relatively large size, but only SVL and shallow dorsal saddles were formally investigated in this study. The short anterior tempo-

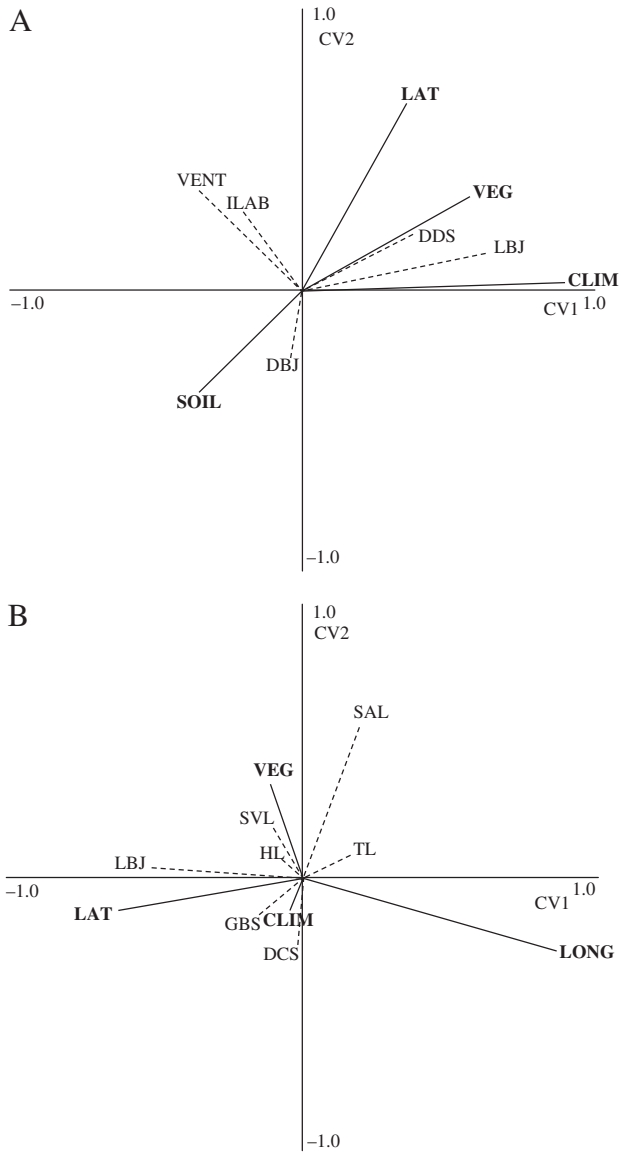

Figure 7. Standardized canonical correlations of the morphological and environmental variables on the first (CV1) and second (CV2) canonical variables for the environmental variables, from the canonical correlation analysis for (A) males and (B) females. Only variables with influential canonical coefficients on CVs 1 or 2 (in bold in Table 7) are shown. Environmental variables are in bold, dashed lines point to morphological variables.

rals was not tested explicitly, but loreal shape as determined by position, shape and relative size of head scalation was not successful at differentiating *etheridgei* from the other subspecies. Shallow dorsal saddles did not prove to be diagnostic, but the male *etheridgei* was 3.1 standard deviations (SD) larger than its most morphologically similar subspecies, *antonii*, in SVL. The female *etheridgei* was 3.6 SD larger than *antonii* females in SVL. According to Mayr (1969), a population may be assigned subspecies status if character divergence is of at least 2.6 SD. The *etheridgei* subspecies was also significantly different in head width, with the male deviating by 3.5 SD from *antonii* in head width and the female by 2.9 SD.

The *lecontei* subspecies did have more dorsal saddles that were shorter in length but did not have more lateral graininess. Klauber's (1941) conclusion that *lecontei* has more lateral graininess likely results from his exclusion of the *clarus* form from the *lecontei* subspecies, a colour morph that generally lacks that characteristic. I found that *tessellatus* had more lateral graininess than *lecontei* as well as more lateral blotching between dorsal saddles. Snout shape in *lecontei* was not significantly different for females, but males had longer, wider snouts than *tessellatus* and shorter snouts than *antonii*. None of the original distinguishing characters for *lecontei* (Klauber, 1941) were diagnostic. The *tessellatus* subspecies was originally characterized as having a lower ventral scale count and upturned snout (Klauber, 1941). Its ventral scale count was significantly lower only for males and only when compared with *lecontei*. Snout shape differed only for males, which had shorter, narrower snouts. Again, neither of these characters was diagnostic.

The ability of the characters examined to consistently differentiate among the subspecies of *R. lecontei* was low overall. No subspecies pair was distinct in all canonical discriminant analyses, and only the linear morphometric variables for the males succeeded in separating all subspecies pairs. This result is interesting, considering that the original characters that distinguished subspecies were primarily based on colour pattern and scalation. The LMV may be so successful at segregating subspecies because of the overall large size of the *etheridgei* form. Removing *etheridgei* from the canonical variable analyses does not affect Mahalanobis distances between subspecies pairs, and canonical coefficients are altered only for the linear morphometric variables for both sexes. SVL, head length and snout length decrease in importance, while tail-length and head width increase in importance. Snout width remains influential with or without *etheridgei*.

In light of this study's results, I recommend subsuming all continental subspecies (*antonii, lecontei* and *tessellatus*) back into *R. l. lecontei* and maintaining *R. l. etheridgei* as a separate subspecies. The arguments for this taxonomic revision are as follows: first, no diagnostic characters were identified for the three continental subspecies; only *etheridgei* was diagnosable by SVL. Second, no continental subspecies met Mayr's (1969) criterion for morphological divergence, while *etheridgei* satisfied it for both SVL and head

width. Third, multivariate statistical analyses were unable to consistently identify any significantly differentiated subspecies pair, leading to the conclusion that a single taxonomic signal does not exist. In addition, morphological characters showed discordant variation with environmental variables, indicating that morphological variation within *R. lecontei* is not strictly categorical in nature.

Despite the general absence of a single taxonomic signal within the species, a word should be said for the unique biogeography of *etheridgei* that underscores the few morphological differences that were identified. The peninsular population of *R. lecontei* closest to Isla Cerralvo is almost 600 km north in Baja California Norte (Stebbins, 1985), so the *etheridgei* population is probably experiencing no gene flow from either Baja California or the Mexican mainland and is therefore likely to be following a unique evolutionary trajectory. The inability of canonical discriminant analyses to consistently distinguish *etheridgei* from the other subspecies may be due to the extremely small sample size available for this study. In the absence of any other formal statistical analyses (see Grismer, 1990, 1999) or information on genetic relationships, *etheridgei* should be maintained as a subspecies rather than elevated to full species (Grismer, 1999).

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APPENDIX

Institutional abbreviations

CAS	California Academy of Sciences, San Francisco
LACM	Los Angeles County Museum, Los Angeles
MVZ	Museum of Vertebrate Zoology, Berkeley
UAZ	University of Arizona, Tucson
UNAM	Universidad Nacional Autonoma de México, México D.F.
UNM	University of New Mexico, Albuquerque

MATERIAL EXAMINED

Rhinocheilus lecontei antonii

Chihuahua, Mexico (MVZ 70999; MVZ 73016; MVZ 73017; MVZ 73018; MVZ 73019), Sinaloa, Mexico (MVZ 59305; MVZ 59306; MVZ 59307; MVZ 59308; MVZ 66205; MVZ 71332; MVZ 180360; MVZ 180361), Sonora, Mexico (MVZ 50737; MVZ 71331; MVZ 75961; MVZ 76370; MVZ 133016; MVZ 133017; MVZ 136783).

Rhinocheilus lecontei etheridgei

Baja California Sur (CAS 98095; UNAM uncatalogued specimen).

Rhinocheilus lecontei lecontei

Baja California Norte, Mexico (MVZ 182121; MVZ 189973), Cochise Co., Arizona (MVZ 44749; MVZ 44750; MVZ66879; MVZ 74267; MVZ 97122; MVZ 97123; MVZ 206966; MVZ 206967; MVZ 206968; MVZ 206969; MVZ 209083; MVZ 209137; MVZ 217642; MVZ 217643; UAZ 14802; UAZ 14803; UAZ 28570; UAZ 32777; UAZ 34678; UAZ 40274; UAZ 40282; UAZ 40884; UAZ 41404; UAZ 41425; UAZ 41431; UAZ 41438; UAZ 41443; UAZ 41448; UAZ 43067; UAZ 43068; UAZ 43069; UAZ 43776; UAZ 43907; UAZ 44282; UAZ 44364; UAZ 45474; UAZ 45556; UAZ 46530; UAZ 46630; UAZ 46631; UAZ 46632; UAZ 47311; UAZ 48226; UAZ 48227; UAZ 49174; UAZ 50014), Graham Co., Arizona (MVZ 49934; MVZ 97120), Maricopa Co., Arizona (MVZ 97119), Mohave Co., Arizona (MVZ 56975; MVZ 63638), Pima Co., Arizona (CAS 80700; CAS 80701; CAS 80728; CAS 80729; CAS 152524; CAS 190459; CAS 190462; CAS MVZ 65725; MVZ 66405; MVZ 78044; MVZ 84455; MVZ 196864; MVZ 209081), Pinal Co., Arizona (CAS 80678; CAS 84123; CAS 190444; MVZ 128215; MVZ 128216; MVZ 128217), Santa Cruz Co., Arizona (CAS 190439; CAS 190440; CAS 190441), Yavapai Co., Arizona (MVZ 80181), Yuma Co., Arizona (MVZ 63636; MVZ 63637), Alameda Co., California (CAS 196186; CAS 196187; CAS 199404; CAS 199405; MVZ 66386), Fresno Co., California (MVZ 94812; MVZ 175838), Imperial Co., California (CAS 182387; CAS 182388; CAS 182389; CAS 182390; CAS 182391; CAS 182392; CAS 182393; MVZ 63632; MVZ 85097), Inyo Co., California (MVZ 3713; MVZ 64122; MVZ 65736; MVZ 65757; MVZ 179940; MVZ 193317; MVZ 193318; MVZ 200862), Kern Co., California (MVZ 6168; MVZ 6834; MVZ 11424; MVZ 56771; MVZ 59990; MVZ 65729; MVZ 65759; MVZ 70345; MVZ 107204; MVZ 164960; MVZ 173033; MVZ 175839; MVZ 182542; MVZ 193312; MVZ 193315; MVZ 193316; MVZ 193319), Lassen Co., California (MVZ 20485), Los Angeles Co., California (MVZ 641; MVZ 36141; MVZ 42405), Madera Co., California (CAS 49222; MVZ 36186), Placer Co., California (MVZ 179991), Riverside Co., California (MVZ 144; MVZ 30269; MVZ 56770; MVZ 61010; MVZ 66399; MVZ 182597), San Benito Co., California (MVZ 6835), San Bernadino Co., California (MVZ 18033; MVZ 26658; MVZ 39616; MVZ 41149; MVZ 56498; MVZ 60806; MVZ 63634; MVZ 63635; MVZ 37274; MVZ 71765; MVZ 85239; MVZ 94811; MVZ 128485; MVZ 134193; MVZ 173034; MVZ 173700; MVZ 176177; MVZ 176120; MVZ 176122; MVZ 182555; MVZ 182556; MVZ 182560; MVZ 193321; MVZ 203667; MVZ 207924; MVZ 207926; MVZ 207927; MVZ 207928; MVZ 214653; MVZ 214654), San Diego Co., California (MVZ 228; MVZ 27023; MVZ 55751; MVZ 58137; MVZ 61098; MVZ 66418; MVZ 179941; MVZ 179942; MVZ 179943; MVZ 179944; MVZ 179945; MVZ 222346; MVZ 222348; MVZ 222349; MVZ 222350; MVZ 222351; MVZ 222352; MVZ 222353; MVZ 222354), San Joaquin Co., California (CAS 161386; CAS 190418; CAS 190419; CAS 198819; MVZ 66735; MVZ 72488; MVZ 134117; MVZ 171761; MVZ 176121; MVZ 180356), San Luis Obispo Co., California (CAS 182407), Santa Barbara Co., California (CAS 182409), Santa Clara Co., California (MVZ 56772), Stanislaus Co., California (MVZ 43655), Sutter Co., California (MVZ 57354), Tulare Co., California (CAS 4279; CAS 162068; MVZ 2754; MVZ 2755), Churchill Co., Nevada (MVZ 42093; MVZ 42094), Clark Co., Nevada (MVZ 20410; MVZ 57602; MVZ 20409; MVZ 56921), Lincoln Co., Nevada (MVZ 14299), Nye Co., Nevada (MVZ 49933; MVZ 97118), Washoe Co., Nevada (MVZ 222355), Kane Co., Utah (MVZ 74197), Washington Co., Utah (MVZ 171542) Hidalgo Co., New Mexico (MVZ 66878; UAZ 32572).

Rhinocheilus lecontei tessellatus

Coahila, Mexico (MVZ 81348), Bernalillo Co., New Mexico (LACM 2646), Dona Ana Co., New Mexico (LACM 2647), Eddy Co., New Mexico (LACM 2648), Hidalgo Co., New Mexico (LACM 133933; UNM 4636; UNM 33799; UNM 37098; UNM 37099; UNM 47601; UNM 49237; UNM 49238; UNM 53347), Luna Co., New Mexico (UNM 312; UNM 54916), Quay Co., New Mexico (LACM 20819), Socorro Co., New Mexico (LACM 20818; LACM 63461; UAZ 4567), Brown Co., Texas (MVZ 43829), Howard Co., Texas (MVZ 38432), Starr Co., Texas (MVZ 53895), Terrell Co., Texas (MVZ 110860; MVZ 110863), Uvalde Co., Texas (MVZ 68453), Webb Co., Texas (MVZ 12705).