MORPHOLOGICAL VARIATION IN *BOLITOGLOSSA VALLECULA* (AMPHIBIA: CAUDATA: PLETHODONTIDAE) IN THE CORDILLERA CENTRAL OF COLOMBIA

VARIACIÓN MORFOLÓGICA EN *BOLITOGLOSSA VALLECULA* (AMPHIBIA: CAUDATA: PLETHODONTIDAE) EN LA CORDILLERA CENTRAL DE COLOMBIA

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Abstract

Body size and head shape variation was quantified in four populations of *Bolitoglossa vallecula* in the Cordillera Central of Colombia. Two of these four populations occurred in sympatry with *B. ramosi*, an ecologically similar salamander species that could potentially compete with *B. vallecula* for food resources. There was no evidence of sexual size or head shape dimorphism in any population, so data for males and females were pooled. The populations differed significantly in mean body size, but this variation was not associated with elevation. Six head shape characteristics associated with head morphology were quantified and analyzed using the ratio of each variable over the geometric median, given that this is the most preferred method in the literature for describing conformation while controlling for the effects of size. One of the populations sympatric with *B. ramosi* (Bello) exhibited significant differences in conformation of head morphology when compared to the allopatric populations, while the other sympatric population did not. While the evidence for character displacement due to ecological competition was equivocal, the analyses highlight areas where further research should be directed.

Key words: character divergence, head morphology, morphological variation, Plethodontidae

Resumen

Cuantificamos la variación en el tamaño y forma del cuerpo y cabeza de *Bolitoglossa vallecula* en cuatro poblaciones de la Cordillera Central de Colombia. Dos de estas cuatro poblaciones ocurren en simpatría con *B. ramosi* una especie de salamandra ecológicamente similar que podría potencialmente competir con *B. vallecula* por recursos alimenticios. En ninguna de las poblaciones encontramos evidencias de dimorfismo sexual en el tamaño o forma de la cabeza, por lo tanto los datos de hembras y machos fueron agrupados. Las poblaciones difirieron significativamente en el tamaño promedio del cuerpo, pero esta variación no estuvo asociada con la elevación. Cuantificamos seis características de la forma de la cabeza asociadas con la morfología de la misma y las analizamos utilizando las proporciones de cada variable sobre la media geométrica, debido a que esta metodología es la más utilizada en la literatura para describir conformación mientras se controla por el efecto del tamaño. Una de las poblaciones simpátricas con *B. ramosi* (Bello) presentó diferencias significativas en la conformación de la morfología de la cabeza cuando se comparó con las poblaciones alopátricas, mientras que la otra población simpátrica no. Aunque en este trabajo la evidencia de desplazamiento de caracteres en respuesta a la competencia ecológica no es concluyente, este análisis destaca áreas de investigación que vale la pena adelantar en el futuro.

Palabras claves: divergencia de caracteres, morfología de la cabeza, Plethodontidae, variación morfológica

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INTRODUCTION

Molecular studies conducted during the last century on plethodontid salamanders resulted in the recognition of large numbers of cryptic species not easily distinguishable using traditional morphological characters, which led to the characterization of this Family as being "morphologically conservative", or as showing "morphological stasis" (Carr 1996, Chippendale et al. 2000, Garcia-Paris et al. 2000, Highton 1989, 1995, Highton and Peabody 2000, Lynch et al. 1983, Wake and Jockusch 2000, Wake et al. 1983). However, multivariate analyses of morphometric data sets have been able to distinguish among species previously defined genetically (Carr 1996, Davis and Pauly 2011) or to detect significant levels of shape variation among populations within species (Adams and Beachy 2001, Adams and Rohlf 2000, Davis and Pauly 2011, Matsui et al. 2011). Some cases of intra-specific variation appear to be due to character displacement, with trophic morphology diverging in populations that occur in sympatry with species that compete with them for food resources (Adams 2000, 2004, Adams and Rohlf 2000, Adams et al. 2007, Arif et al. 2007).

In this study, we used multivariate morphometric analyses to compare four populations of the plethodontid salamander *Bolitoglossa vallecula* (Brame and Wake 1963) in the Cordillera Central of Colombia. Our goal was to document levels of morphological variation and sexual dimorphism in and among these populations, as well as inspect for evidence of character displacement in head morphology. The distribution of *B. vallecula* overlaps slightly with the distribution of *B. ramosi* (Wake and Brame 1972), another plethodontid salamander also in the *adspera* species group (Parra-Olea et al. 2004). Both species occur from 1200 to 3000 m elevation, are nocturnal, and prefer similar micro-habitats of herbaceous and shrubby vegetation up to 1 m high along the margins of streams and in forest interiors (Páez et al. 2002, Palacio et al. 2006), suggesting that inter-specific competition might be important where the two species occur in sympatry. Two of the populations of *B. vallecula* we examined in this study occurred in sympatry with *B. ramosi*, and the remaining two did not.

MATERIALS AND METHODS

Collecting material. Four collecting trips were conducted from 17 July 2008 to 26 January 2009 in the department of Antioquia (Colombia); one each to two allopatric populations and two sympatric populations of *B. vallecula* (figure 1). The allopatric populations were located in the municipality of **Yarumal** (corregimiento Llanos de Cuivá, 06º 51' 06" N, 75º 29' 30" W, 2637 m) and the municipality of **Belmira** (vereda Los Patos, 06º 38' 29" N, 75º 39' 55" W, 2941 m). The populations sympatric with *B. ramosi* were located in the municipality of **San Pedro** de los Milagros (vereda La Lana, 06º 28' 23" N, 75º 37' 17" W, 2746 m) and the municipality of **Bello** (corregimiento San Felix, 06º 21' 12" N, 75º 38' 30" W, 2597 m). Individuals were hand captured, sacrificed with topical application of 5% xilocaine, fixed in 10% formalin, preserved in 70% ethanol, and deposited in the Museo de Herpetología of the Universidad de Antioquia (MHUA 5984-MHUA 6010, MHUA 6038, MHUA 6041, MHUA 6043, MHUA 6046, MHUA 6051, MHUA 6053-MHUA 6055, MHUA 6057, MHUA 6070, MHUA 6072, MHUA 6083, MHUA 6147- MHUA 6161, MHUA 6177, MHUA 6179-MHUA 6182, MHUA 6186, MHUA 6187, MHUA 6190, MHUA 6195, MHUA 6196, MHUA 6198, MHUA 6200, MHUA 6202, MHUA 6204-MHUA 6207, MHUA 6209-MHUA 6214).

Morphological data. For each individual, seven morphological variables were measured according to Carr (1996, figure 2): **1)** snout vent length **(SVL), 2)** head width **(HW), 3)** head length **(HL), 4)** head depth **(HD),** distance between the eye and nares
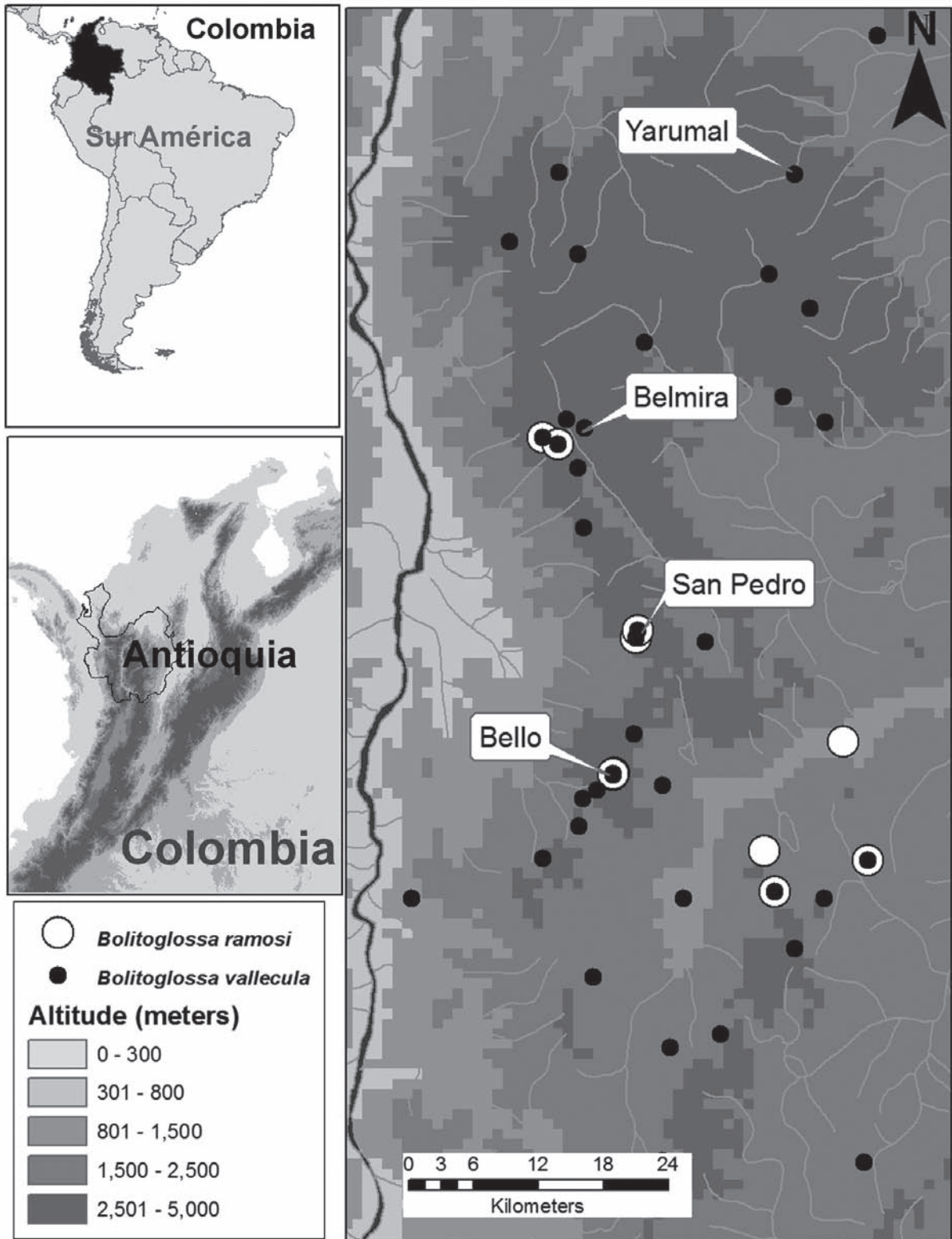
Figure 1. Map showing the known ranges of *B. vallecula* and *B. ramosi* in the Antioquia department of Colombia, with the four study sites indicated

(EN), 6) jaw length **(JL),** and **7)** gape width **(GW).** The six head characters were selected for their widespread use in other studies of salamanders for describing general head morphology (Adams 2000, Carr 1996, Emerson 1985, Fraser 1976a, b). Each measurement was recorded by NS-G on the right side of the individual using a digital caliper (± 0.01 mm precision).
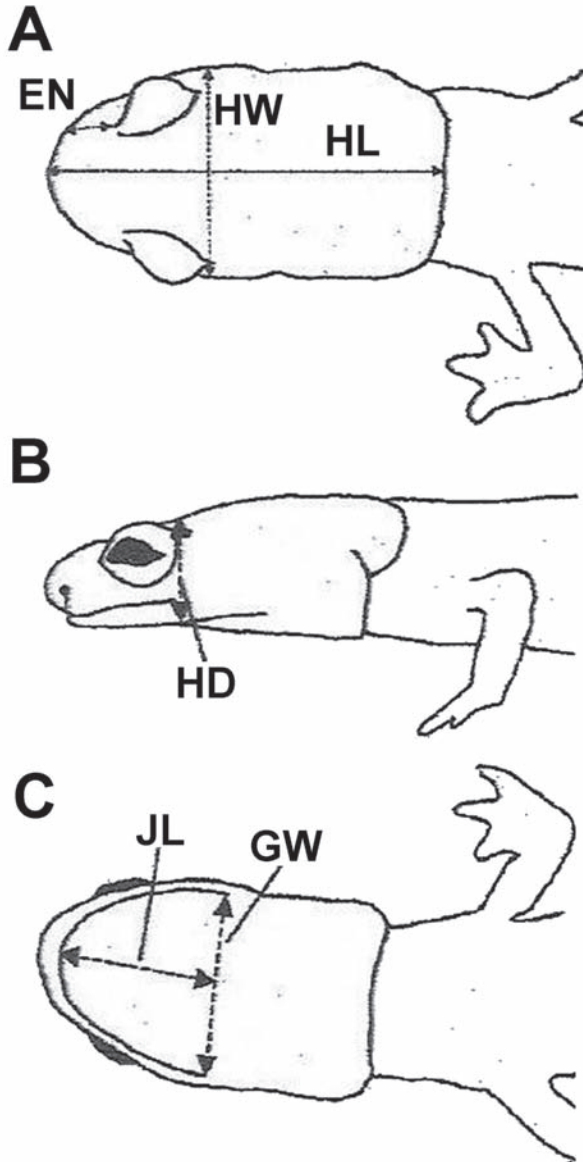
Figure 2. Morphological head characters quantified. **A** = dorsal perspective; **B** = lateral perspective; **C** = ventral perspective [**EN** = distance between the eye and nares; **HW** = head width; **HL** = head length; **HD** = head depth; **JL** = jaw length; **GW** = gape width; from Adams (2000)]

Male *B. vallecula* lack mental glands, so after measuring an individual, it was sexed by opening its abdominal cavity to permit inspection of gross internal anatomy. Males could be differentiated from females by the presence of conspicuous testicles that together constituted approximately 5% of total body volume. To corroborate these determinations, we removed the right reproductive tracts (ovaries and oviduct for females and testicles and epididymis for males) from a sample of individuals. These tissues were fixed in Bouin's solution, embedded in paraffin, sectioned into 3 μm layers, and stained with hematoxylin-eosin. These histological preparations confirmed the sexes previously determined by inspection of gross anatomy in all cases.

Statistical analysis. To inspect for differences in body size among populations, we first compared the SVLs of males and females from each population using t-tests on natural log transformed values. Because none of the populations exhibited significant sexual size dimorphism (see results), male and female data for each site were pooled and the four populations were compared using ANOVA and Tukey *post-hoc* tests.

Given that other morphological measures are strongly correlated with overall body size, variation in these characters may only reflect variation in overall size, but not shape, complicating the interpretation of patterns detected by univariate analyses (Adams 2000). Various methods, with their own particular strengths and weaknesses, have been employed to control for size in the study of shape variation (Burnaby 1966; dos Reis et al. 1990, Humphries et al. 1981, Lleonart et al. 2000, Mosimann 1970, Mosimann and James 1979, Thorpe 1976). In this study, to control for size in the analysis of shape variation in the head variables, all measures were natural logarithm transformed before applying the method recommended by Mosimann (1970), given that its geometric

interpretation of shape seems the most appropriate for use with hypotheses relating to functional biology (Butler and Losos 2002). This method eliminates the effect of size on each measurement employing an index measured directly from the size of the individual: the geometric mean (the fourth root of the product of the variables). Thus, size is the geometric mean **(GM)** of the variables and the ratios of the logarithm of each variable over the geometric mean [log (variable/GM) = log (variable)-log (GM)] are used as the shape data set (Butler and Losos 2002, Mosimann 1970, Mosimann and James 1979).

Another complication in comparisons of head morphology among populations is the existence of sexual shape dimorphism. To inspect for this possibility, variation in head shape of males and females in each *B. vallecula* population were compared by means of a non-parametric multivariate analysis of variance (NPMANOVA), using Euclidian distance as a measure of dissimilarity (Gutiérrez and Molinari 2008). Significance levels for the NPMANOVAs were calculated via a permutations test with 10,000 replicates. To visualize variability patterns of males and females, a principal components analysis of the variance-covariance matrix was conducted and plots of the first two components were compared. Given that no differences among sexes were detected (see results below; figure 3), data from males and females were pooled for each population for subsequent analyses.
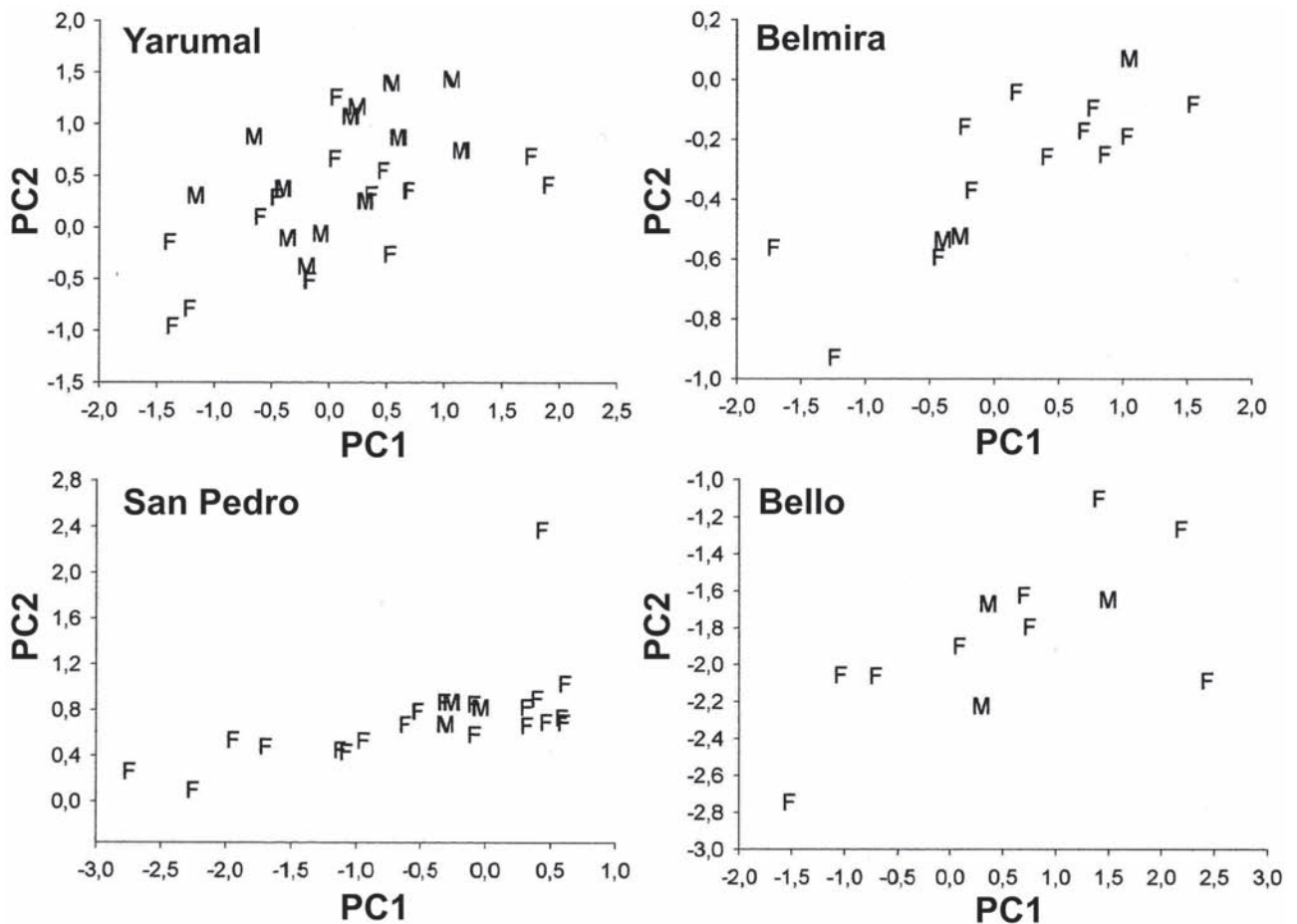

Figure 3. Principal components (PC) plots of head conformation for males (M) and females (F) for each population

To compare patterns of variation in head shape among populations, a NPMANOVA test was employed with Euclidian distance again as the measure of dissimilarity (Gutiérrez and Molinari 2008), and with significance levels calculated as described above. In addition, a permutation test with 10,000 replicates was conducted to test the equality of the measurements. Mahalanobis distances (D^2) were calculated to quantify the similarity of two multidimensional variables, considering the variation and the correlation among them (Slice et al. 1998). To visualize the differences and similarities among populations, a principal components analysis was conducted using the variance-covariance matrix to generate plots of the first two principal components. A discriminant analysis also was conducted to confirm or reject whether two populations were morphometrically distinct, where the linear functions of the variables employed were used to describe the differences among groups (Renchen 2002), to classify individuals as pertaining to a sympatric or allopatric population, expressed in terms of the percent correct classification of the specimens (Hammer et al. 2001).

All statistical analyses were evaluated using a significance level of $\alpha = 0.05$ and were conducted using the software PAST 1.89 (Hammer et al. 2001).

RESULTS

In the allopatric populations of *B. vallecula*, 27 individuals were collected from Yarumal (14 females and 13 males) and 15 individuals were collected from Belmira (12 females and three males). In the sympatric populations, 23 individuals were collected from San Pedro (20 females and three males) and 12 individuals were collected from Bello (nine females and three males).

There was no evidence of sexual body size (SVL) dimorphism in the four populations (Yarumal, t = 0.49, p > 0.10; Belmira, t = 0.22, p > 0.10; San Pedro, t = 0.42, p > 0.10; Bello, t = 0.95, p > 0.10). Mean body sizes of the four populations (male and female data pooled) differed (LnSVL, $F_{3, 73} = 13.73$, p < 0.001), with individuals from the Bello population significantly smaller than individuals from the remaining three sites and individuals from the San Pedro population significantly larger than the remaining three sites (Tukey post-hoc test).

No significant sexual dimorphism in head shape was detected in the four sites (Yarumal $F_{1, 25} = 0.79$, p > 0.10; Belmira $F_{1, 13} = 0.02$, p > 0.10; San Pedro $F_{1, 21} = 0.20$, p > 0.10; Bello $F_{1, 10} = 0.10$, p > 0.10; figure 3), so male and female data were pooled for each locality for the remaining analyses. The percent of total variation explained by the first two principal components for Yarumal was 94.44%, for Belmira was 96.05%, for San Pedro was 96.60%, and for Bello was 94.98%, with the original variable EN loading most heavily on the first principal component in all four populations.

The NPMANOVA comparison of the allopatric populations vs. the sympatric populations showed significant head morphology differences $(F_{1, 75} = 8.17$, p < 0.001), while the permutation test did not (Mahalanobis distance = 0.0924, p > 0.10). The principal components analysis revealed two groups; one comprised of the two allopatric populations and San Pedro and the other comprised of individuals from the other sympatric population of Bello (figure 4). The first principal component explained 71.20% of the total variation and the second (shape) principal component explained 22.05%. The original variables that contributed most to the separation of the groups were HL and EN, both in the second principal component. The discriminant analysis that attempted to classify individuals with respect to their population based upon the principal components analysis achieved a correct classification rate of 71.43%.
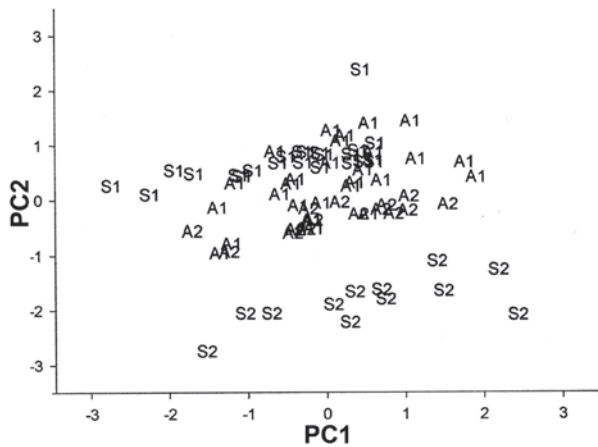
Figure 4. Principal components **(PC)** plot of head conformation in the four populations of *B. vallecula* (sexes pooled). **A1** (Allopatric 1) = Yarumal; **A2** (Allopatric 2) = Belmira; **S1** (Sympatric 1) = San Pedro; **S2** (Sympatric 2) = Bello

An NPMANOVA did not reveal differences in head conformation among the two populations of *B. vallecula* that occur in allopatry (Yarumal and Belmira; $F_{1, 40}$ = 1.68, p > 0.10), but the permutation test was significant (Mahalanobis distance = 0.4867, p < 0.001). The two populations were not clearly separated in the plot of the first two principal components, with the first component explaining 79.03% of the total variation, and the second component only 11.89%. The original variable with the largest loading on the first principal component was EN, and HL loaded most heavily on the second principal component.

The two sympatric populations of *B. vallecula* exhibited significant differences in head conformation ($F_{1, 33}$ = 18.94, p < 0.001), which was corroborated with the permutation test (Mahalanobis Distance = 1.638, p < 0.001). These first two components of the principal component analysis explained 96.56% of the total variation (first component = 70.59%, second component = 25.97%). The original variables that contributed most to the separation of these two populations were HL on the first component and EN and HL on the second component.

DISCUSSION

The Bello population of *B. vallecula* differed from the other three populations in both body size and head morphology. A meta-analysis by Ashton (2002) of studies on body size variation in amphibians suggested that most amphibian species conform to Bergmann's rule (Bergmann 1847), with larger mean body sizes expressed in populations that experience cooler climatic conditions. However, in this study, the population with the largest mean body size was not Belmira (the site with the highest elevation), and the population with the smallest mean body size (Bello) differed significantly from another site (Yarumal) that was only 100 m higher in elevation. A more extensive meta-analysis by Adams and Church (2008) that included more studies of salamander species in the data base failed to show evidence that amphibian populations follow Bergmann's rule. Thus, the significant variation among populations in mean body size demonstrated in this study, conducted over just a 450 m elevational gradient, probably is not related to climatic differences, or at least to temperature differences, among the sites.

The Bello population not only differed from the others in terms of mean body size, but also in terms of its head morphology. Differences were mainly in terms of HL and EN, two original variables that might be related to prey capture or handling. Thus, the shape differences in the Bello population could be related to the fact that there exists a high density of *B. ramosi* individuals in this small forest fragment (39 individuals were observed), implying that the divergence of *B. vallecula* morphology could represent a response to competition with *B. ramosi* (Brown and Wilson 1956, Dayan and Simberloff 2005, Hespenheide 1973) for resources (Maret and Collins 1997, Schluter and McPhail 1993, Smith 1990). Under this scenario, selection at this site has favored individuals with modified phenotypes thanks

to the reduction in competition this confers, but with a trade-off in terms of lowered fitness, when compared to the allopatric populations or the sympatric populations where *B. ramosi* densities are not as high (Pfenning and Pfenning 2005). Cranial conformation thus may be a good phenotypic indicator of character displacement in response to inter-specific competition (Myers and Adams 2008), although to confirm this, additional data on diet composition, micro-habitat preferences, and geographic ranges are needed to permit consideration of all dimensions of the fundamental niche (Hespenheide 1973, Pianka 1969).

Alternatively, the differences in cranial morphology at the Bello site might be unrelated to resource competition. Differences in the head shape at Bello may simply reflect other genetic or environmental variation that differentially affects developmental processes there (Carr 1996). Marked morphological variation among populations of salamanders often exists in many characters, with levels of intra-specific variation often comparable to the magnitude of inter-specific variation (Alberch 1981, Alberch and Alberch 1981, Carr 1996, Garcia-Paris et al. 1998, Jaekel and Wake 2007), due to extreme morphological and ecological homoplasy (Parra-Olea and Wake 2001).

While this study did not provide unequivocal evidence for character displacement in head shape in *B. vallecula* when in sympatry with *B. ramosi*, the morphological variation documented does argue that further study is warranted. For example, the variable that most distinguished Bello individuals from those of the other populations was HL, which might be related to tongue length or the muscles involved in its protrusion. Unlike other plethodontid salamanders, individuals in the genus *Bolitoglossa* obtain their prey by protruding their tongue, so perhaps the other variables measured in this study to quantify

cranial conformation are not as directly linked to diet as they are in other plethodontid species.

To fully understand the processes that have generated and are maintaining the intra-specific morphological differences documented in this study, it will be necessary to evaluate whether differences in head (trophic?) morphology also exist among *B. ramosi* populations, compare diets of individuals of both species at the different sites, and quantify additional traits potentially related to other niche dimensions, such as humerus, femur, and trunk lengths (Adams and Beachy 2001) or foot morphology (Jaekel and Wake 2007). We also recommend using geometric morphological methods in future studies, so that the geometry of structures may be captured and differences in morphology expressed in terms of a continuum (Rohlf and Marcus 1993), thereby considering the geometric relationships among measures and preserving this information during the analyses (Adams et al. 2004).

ACKNOWLEDGEMENTS

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