

Effect to age on the geographic variation in morphometric traits among populations of *Boana cordobae* (Anura: Hylidae)

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Abstract: The morphometric variation of body size is an important topic of the natural history of the species which has been received particular interest. In this study, we estimated differences in body size and age structure of six populations of *Boana cordobae* living at different altitudes, 808-2 310 m.a.s.l. in Córdoba and San Luis provinces (Argentina). We measured 15 morphometric variables and used skeletochronology to age determination of 79 individuals. Morphometric variables showed significant differences between sexes, being females larger than males, even when the effect of age was taken into account. We found a significant relationship between age and most of the morphometric variables. When removing the effects of age, we found significant inter-population differences in body size. Males from the high-elevation populations were larger than individuals from low-elevation populations. These results suggest that a difference in age structure between populations is a main factor for the geographic variation in body size of this species. Rev. Biol. Trop. 66(4): 1401-1411. Epub 2018 December 01.

Key words: *Boana cordobae*; skeletochronology; morphometric variables; altitudinal variation.

Morphometric variation in body size among specimens has been of particular interest since serving support to explain evolutionary and ecological phenomena, because it is an important life-history trait influencing many aspects of an individual's biology and is indispensable for the description of new species (Roff, 1992; Lee, 1982; Bernal & Clavijo, 2009). Besides, intraspecific variation in body size is particularly intriguing because it suggests strong associations between an organism's size and its environment (Angilletta & Dunham, 2003).

In amphibian species, with a broad geographical distribution, show a high phenotypic plasticity in adult body size (Angilletta, Steury, & Sears, 2004; Tood, Scott, Pechmann, &

Gibbons, 2011; Özdemir et al., 2012). The most widely recognized generalization for body size is Bergmann's rule: the observation that within species or among closely related species, endothermic and some ectothermic vertebrates tend to be larger in relatively cool climates (Ashton, 2002; Angilletta et al., 2004; de Queiroz & Ashton, 2004); and this theory as well has been widely supported in amphibians (Olalla-Tárraga & Rodríguez, 2007; Ma, Tong, & Lu, 2009; Liao, Lu, Shen, & Hu, 2010a; Liao & Lu, 2010). The impact of environmental gradients, caused by for altitude, mainly in mean air temperature can influence life-history of ectothermic organisms (Lu, Li, & Liang, 2006; Ma et al., 2009; Iturra-Cid, Ortiz, & Ibagüen-goytia, 2010; Lou, Jin, Liu, & Mi, 2012), as

age and growth rate (Miaud, Guyétant, & Elmerberg, 1999; Cvetković, Tomašević, Ficaretola, Crnobrnja-Isailović, & Miaud, 2009; Iturra-Cid et al., 2010). Numerous studies suggest that at higher altitude with lower temperatures, organisms show slower growth rates and later age at sexual maturity, therefore larger body size, because the body size is determined by a combination of age and growth (Iturra-Cid et al., 2010; Liao & Lu, 2010; Liao, Zhou, Yang, Hu, & Lu, 2010b; Lou et al., 2012; Li et al., 2013).

Age determination and body size in anurans are important characteristics of life-history traits of species (Miaud et al., 1999; Liao, & Lu, 2011; Mao, Huang, Mi, Liu, & Zhou, 2012; Mi, 2015). Because anurans show indeterminate growth is expected that body size and age are positively correlated (Halliday & Verrell, 1988; Duellman & Trueb, 1994). However, this relationship is controversial: in most anurans, the age of adult positively correlated with their size (Liao & Lu, 2010; Ashkavandi, Gharzi, & Abbassi, 2012; Altunişik & Özdemir, 2013; Otero et al. 2017a; Otero, Valetti, Bionda, Salas, & Martino 2017) while in others this correlation is weak or non-existent (Leclair, Leclair, Dubois, & Daoust, 2000; Pancharatna & Deshpande, 2003; Fattah, Slimani, El Mouden, Grolet & Joly, 2014). As reported by many authors, comparisons in age, growth and body size are particularly useful when geographic variations in life-history traits are studied (Lu et al., 2006; Liao & Lu, 2010; Liao, Lu, Shen, & Hu, 2011; Liu, Liu, Huang, Mi, & Li, 2012).

Boana cordobae (Barrio, 1965), endemic to Argentina, is one of the most common anuran species breeding in rivers and streams from Córdoba and San Luis provinces (Barrio, 1965; Cei, 1980; Baraquet, Salas, & Martino, 2013). Although age, growth, sexual size dimorphism (Otero et al., 2017a) and geographic variations in morphometric variables (Baraquet, Grenat, Salas, & Martino, 2012) have reported in recent years, there is no information about relationships between morphometric variables and age of the individuals along a altitudinal range. Since in that different altitudes represent

different environmental conditions, we hypothesized that life-history traits among populations will be different, with values of age and morphometric variables greater at high-altitude. In the present study, we reanalyzed the data used by Baraquet et al. (2012) incorporating age as a covariate, with the objective of: 1) test whether age and body size are correlated, 2) confirm whether age and morphometric variables vary with altitude, 3) and describe the altitudinal variation in morphometric variables of six *B. cordobae* populations from different elevation using age as covariate.

MATERIALS AND METHODS

Study area: We collected individuals of *B. cordobae* between September 2006 and May 2011 from six populations of Córdoba and San Luis Provinces (Argentina), covering an area of about 20000 km² and an altitudinal range between 800 and 2300 m.a.s.l.: Achiras (808 m.a.s.l., 33°09'28.64" S - 64°58' 55.13" W), Las Guindas (930 m.a.s.l., 32°35'35.22" S - 64°42'38.92" W), La Carolina (1634 m.a.s.l., 32°48'43.94" S - 66°05'48.15" W), Los Tabaquillos (2107 m.a.s.l., 32°23'59.75" S - 64°55' 33.69" W), Pampa de Achala (2150 m.a.s.l., 31°49'41.8" S - 64°51'44.9" W), Los Linderos (2310 m.a.s.l., 32°00'54.05" S - 64°56'42.97" W) (Baraquet et al., 2012).

Field data collection: We hand-captured individuals during surveys at streams and ponds edges. We anesthetized the frogs by immersion in MS-222 (0.3 %) (Green, 2018). For each individual, we determined the sex of adults using external secondary sexual characters (vocal sacs in males and readily visible eggs through the ventral skin of females). For each individual, we measured 15 morphological variables, using a digital caliper (Cei, 1980, Heyer, Rand, Gonçalves da Cruz, Peixoto, & Nelson, 1990; Martino & Sinsch, 2002): snout-vent length (SVL), head width (HW), head length (HL), eye-snout distance (ESD), inter-nostril distance (IND), inter-orbital distance (ID), eye-nostril distance (END), rostronostril

distance (RND), eye diameter (ED), arm length (AL), femur length (FL), tibia length (TL), foot length (FoL), length third finger (TF), length of the fourth toe (LF4). We clipped the longest right toe of each frog and preserved it in 70 % ethanol. Antifungal or antibacterial and healing agent were added at the puncture to prevent infections (Green, 2018). This procedure was approved by and the Ethical Committee of Investigation of the National University of Río Cuarto (file number 38/11). Each individual was released 2 hr later into their places of capture.

Age determination: We performed laboratory protocols following standard skeletochronology methods (Bionda et al. 2015; Otero et al. 2017a), cross sectioning of the diaphysis at 10-12 μm using a rotary microtome and staining with Ehrlich's haematoxylin (3 min).

One lines of arrested growth (LAGs) correspond to each year of life; this assumption is based on Sinsch (2015) for neotropical amphibian species inhabiting environments with high seasonality. LAGs were independently counted by two researchers, using a light microscope Zeiss Axiophot-Axiolab (100X) equipped with digital camera Canon G10, software Axio Vision 4.3.

We identified double and false lines following Liao and Lu (2010a) and assessed endosteal resorption based on the presence of the Kastschenko line (KL; the interface between the endosteal and periosteal zones; Rozenblut & Ogielska, 2005). Endosteal resorption was also confirmed following the protocol of Lai, Lee, & Kam (2005) comparing the major axis of the innermost LAG of each section with the mean of juveniles' LAG without resorption. When the major axis of the innermost LAG of an adult was significantly greater than the mean LAG of juveniles without resorption, we considered that the resorption had occurred.

Analysis: we calculate descriptive statistics for each morphometric variable and age, and used Shapiro-Wilk tests to analyzed normality of distribution. To compare between

males and females in age we used generalize linear mixed models (GLMMs) with the Helmert contrast treating age as a dependent variable and sex as a fixed factor. We used Pearson's correlation coefficient to determine relationships morphometric variables and age; and in morphometric variables between sexes were tested using general linear models (GLMs) with the Helmert contrast treating each morphometric variables as a dependent variable, altitude as a fixed factor and age as a random factor (covariable). We also estimate the sexual size dimorphism using the Lovich and Gibbons (1992) sexual dimorphism index (SDI): $\text{SDI} = (\text{size of larger sex} / \text{size of smaller sex}) \pm 1$, + 1 if males are larger or - 1 if females are larger, and the result arbitrarily defined as positive when females are larger than males and negative in the contrary.

Because the number of females per population is low, we used only data from males for comparison among populations of *B. cordobae*. Descriptive statistics were calculated for different age classes to observed variance. We compared the six populations using Pearson's correlation to determine relationships between morphometric variables and age, and between age and altitude. Differences in mean adult age among populations were also tested using GLMMs and Bonferroni post hoc multiple comparisons. Principal component analysis (PCA) on 15 morphometric variables of all individuals was used to estimate the pattern of correlation and covariation among variables. The principal component with the variables of greater weight were then treated by general linear models (GLM) with PC as a dependent variable, altitude as a fixed factor and age as a random factor (covariable).

Linear regressions between morphometric variables and age were performed to assess the effect of age on morphometric variables. For all variables that were significantly correlated with age, residuals were used to standardize morphometric variables to expected values at an age of 4 years (average age of all males). The standardized morphometric data was analyzed using discriminant analysis, to determine

whether the overall structure of the morphometric variables varied among populations.

We performed all tests using InfoStat (Di Rienzo, 2018) and Statgraphics Centurion XVI.I. All probabilities were two-tailed, and the significant level was set at $\alpha = 0.05$. Means were given \pm SD.

RESULTS

Skeletochronology: The breeding age for 79 individuals (67 adult males and 12 adult females) of *B. cordobae* was successfully determined by mean skeletochronology. Endosteal resorptions did not cause any serious interpretation problems concerning age estimation. Double and false LAGs were observed, but did not affect age assessment.

Age structure: age mean was 3.66 ± 1.23 years (2-7, $N = 67$) for males; and 3.33 ± 1.30 years (2 - 7, $N = 12$) for females. Individuals with three and four years were the most abundant. There was no significant difference in average age between the sexes ($F_{78} = 0.78$, $P = 0.38$). Age at sexual maturity was two year and

maximum longevity was seven years for both males and females (Fig. 1).

Sexual variation in adult age and morphometric variables: Pearson correlation showed that age and most of the measured morphometric variables are correlated ($P < 0.05$) (only three do not correlate with age: ESD ($r = 0.14$, $P = 0.22$), END ($r = 0.19$, $P = 0.09$) and RND ($r = -0.10$, $P = 0.36$). Differences between males and females were compared by general linear models (GLMs, with age as a covariate). From the 15 variables measured, 13 showed differences between the sexes ($P < 0.05$). Females were larger than males, except in END ($F_{78} = 2.15$, $P = 0.12$) and RND ($F_{78} = 0.31$, $P = 0.58$). This too confirm with the sexual dimorphism index (SDI) in body size of 0.095. In specimens of age 2 - 3 - 4 and 7 years, where sample size allowed estimation, variation in SDI was (age of specimens/SDI): 2/0.09, 3/0.08, 4/0.17, 7/0.04 SVL; females were larger than males in average SVL for all ages.

Because the low number of females, and that most of the variables shown significant difference between males and females, later

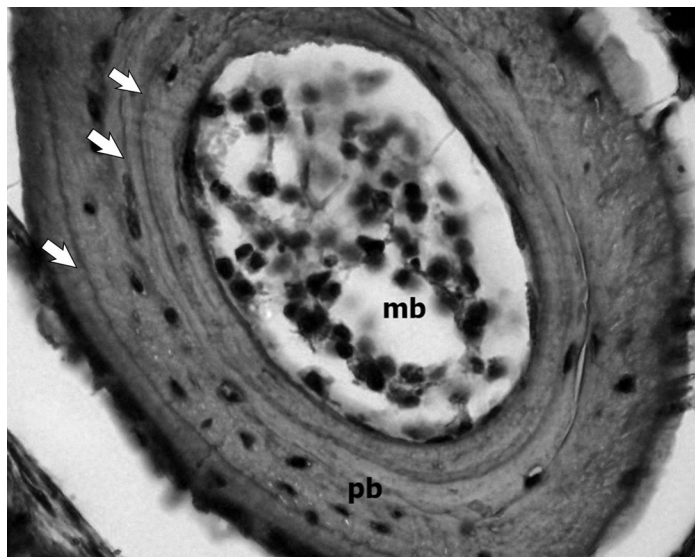


Fig. 1. Examples of phalangeal cross-sections (10 μ m thick) of *Boana cordobae*. Male, SVL: 50.36 mm; 3- year-old. White Arrows = lines of arrested growth (LAGs); mc = medullary cavity; pb = periosteal bone.

analyses were carried out only by taking account of the males.

Age and morphometric variables relationships: The variance of the age class in SVL, and in all variables, was the largest in three-year-old males, with a range of 13.88 mm and a standard deviation of 3.54 mm (SVL = 49.84 ± 3.54 mm); and the smallest reproductive male was 41.30 mm. Body length (SVL) showed considerable variation within some age groups, however age and body size were correlated ($r = 0.38$, $P = 0.0017$). Largest individuals were almost always the oldest ones. The same was observed for most of the morphometric variables (only three variables were not correlated with age, ESD: $r = 0.19$, $P = 0.1089$; ID: $r = 0.22$, $P = 0.08$; RND: $r = -0.15$, $P = 0.22$).

Altitudinal variation in adult males, age and morphometric variables: Pearson correlation show that age and altitude are not correlated ($r = 0.03$, $P = 0.78$) (Table 1). However, average age differs significantly among the six populations (GLMMs $F_{66} = 4.17$, $P < 0.05$), and

post-hoc multiple comparisons indicated that the population of at lower altitudes (808m in elevation) differed with other populations, with older average ages; the populations of intermediates altitudes (930, 1634, 2107 m.a.s.l.) do not differ among them, but with the others, mainly with populations at higher altitudes (2150 and 2310 m.a.s.l.) (Bonferroni post hoc test, $P < 0.05$).

When the effect to age was controlled, partial correlation show that six morphometric variables were not correlated with altitude (ESD: $r = 0.24$, $P = 0.05$; ID: $r = -0.15$, $P = 0.25$; RND: $r = -0.16$, $P = 0.19$; ED: $r = 0.0035$, $P = 0.98$; TL: $r = 0.14$, $P = 0.26$; FoL: $r = 0.21$, $P = 0.09$); while nine were positively correlated with altitude of each population (SVL, HW, HL, IND, END, AL, FL, TF, LF4: $P \leq 0.05$), indicating a clinal trend: males at higher altitudes are significantly larger than those at lower altitudes.

The results of PCA indicate that the first three principal components explain 76.64 % of the variation in the original variables, since the three components showed eigenvalues greater

TABLE 1
Means \pm standard deviation of age and morphometric variables of adult males of *B. cordobae* from six populations

| | Los Linderos 2310 m.a.s.l. | Pampa de Achala 2150 m.a.s.l. | Los Tabaquillos 2107 m.a.s.l. | La Carolina 1634 m.a.s.l. | Las Guindas 930 m.a.s.l. | Achiras 808 m.a.s.l. |
|-----|----------------------------------|-----------------------------------|----------------------------------|------------------------------------|------------------------------------|-----------------------------------|
| Age | $n = 5$ 4.6 ± 1.82 3-7 | $n = 9$ 3.67 ± 0.71 2-4 | $n = 5$ 3 ± 1 2-4 | $n = 12$ 3.33 ± 1.15 2-6 | $n = 26$ 3.27 ± 0.83 2-5 | $n = 10$ 4.9 ± 1.45 3-7 |
| SVL | 56.09 ± 2.89 | 49.43 ± 3.32 | 49.59 ± 1.39 | 51.18 ± 3.23 | 47.04 ± 2.51 | 53.43 ± 2.40 |
| HW | 15.91 ± 1.15 | 14.24 ± 0.71 | 15.35 ± 0.61 | 15.02 ± 0.75 | 13.62 ± 0.92 | 15.61 ± 0.75 |
| HL | 17.36 ± 1.30 | 15.72 ± 0.76 | 15.57 ± 0.71 | 16.11 ± 1.32 | 14.70 ± 1.14 | 16.61 ± 0.98 |
| ESD | 7.42 ± 0.59 | 6.37 ± 0.43 | 6.80 ± 0.48 | 6.89 ± 0.72 | 6.09 ± 0.59 | 7.23 ± 0.32 |
| IND | 4.55 ± 0.39 | 4.15 ± 0.30 | 4.26 ± 0.22 | 4.20 ± 0.39 | 3.70 ± 0.39 | 4.43 ± 0.25 |
| ID | 5.8 ± 0.53 | 5.02 ± 0.28 | 5.27 ± 0.41 | 5.55 ± 0.42 | 5.45 ± 0.77 | 5.76 ± 0.48 |
| END | 4.47 ± 0.43 | 4.15 ± 0.11 | 3.93 ± 0.37 | 4.27 ± 0.52 | 3.57 ± 0.45 | 4.22 ± 0.33 |
| RND | 3.28 ± 0.23 | 2.79 ± 0.46 | 3.24 ± 0.55 | 3.32 ± 0.53 | 3.16 ± 0.43 | 3.39 ± 0.36 |
| ED | 5.86 ± 0.45 | 5.09 ± 0.29 | 4.86 ± 0.10 | 4.82 ± 0.47 | 5.07 ± 0.36 | 5.52 ± 0.26 |
| AL | 27.84 ± 0.94 | 24.46 ± 1.27 | 22.74 ± 0.86 | 24.48 ± 1.98 | 22.21 ± 1.80 | 25.91 ± 1.55 |
| FL | 28.75 ± 0.84 | 25.84 ± 1.18 | 25.77 ± 0.80 | 26.37 ± 1.27 | 23.29 ± 1.93 | 28.18 ± 1.37 |
| TL | 27.81 ± 1.02 | 24.94 ± 1.46 | 24.71 ± 0.69 | 25.86 ± 1.50 | 23.89 ± 1.86 | 28.02 ± 1.36 |
| FoL | 37.54 ± 4.93 | 35.71 ± 1.79 | 35.24 ± 2.28 | 36.59 ± 2.04 | 33.31 ± 3 | 38.49 ± 1.27 |
| TF | 12.49 ± 0.70 | 10.68 ± 0.75 | 9.46 ± 1.27 | 10.31 ± 1.17 | 9 ± 0.92 | 11.05 ± 0.72 |
| LF4 | 17.12 ± 1.13 | 16.08 ± 1.57 | 13.26 ± 1.61 | 14.34 ± 2.39 | 12.41 ± 1.38 | 14.80 ± 1.18 |

or equal to 1.0. PC1 catches mostly variation in measurements concerning to measures related to the body (SVL, HW, HL, AL, FL, TL, FoL, TF, LF4) and the width and length of the head (HW, HL, END), whereas PC2 (RND, ED) and PC3 (ESD, IND, ID) reflects variation in the other variables related to the head (factor loadings summarized in Table 2).

The six studied populations differ significantly in factor scores of PC1 as well as PC2 (GLMs, $P = < 0.001$). Figure 2 shows that PC1 separates mainly the populations at higher and lower altitudes (2310 m elevation and 808 m elevation), and post-hoc tests revealed that males of these two populations were larger than the other populations, and at intermediate

TABLE 2
Principal component analysis. Factor loading matrix in the first three components

| Endpoints | Eigenvectors | | |
|-----------|---------------|--------------|--------------|
| | PC1 (61.23 %) | PC2 (8.42 %) | PC3 (6.99 %) |
| SVL | 0.31* | -0.02 | 0.06 |
| HW | 0.29* | 0.05 | 0.03 |
| HL | 0.27* | -0.16 | -0.19 |
| ESD | 0.25 | 0.27 | -0.28* |
| IND | 0.25 | 0.14 | -0.36* |
| ID | 0.13 | 0.34 | 0.73* |
| END | -0.19 | 0.24* | -0.09 |
| RND | 0.05 | 0.75* | -0.30 |
| ED | 0.21 | 0.32* | 0.13 |
| AL | 0.30* | -0.09 | 0.04 |
| FL | 0.31* | -0.051 | 0.04 |
| TL | 0.30* | -0.01 | 0.21 |
| FoL | 0.29* | -0.06 | 0.21 |
| TF | 0.29* | -0.16 | -0.07 |
| LF4 | 0.25* | -0.22 | -0.12 |

The asterisk indicates variables that most strongly correlated with respective principal component.

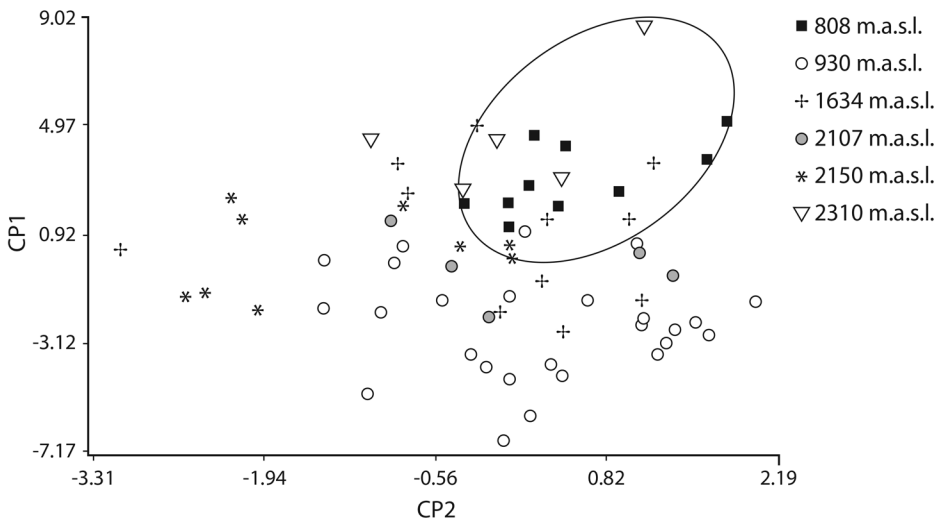


Fig. 2. Scatterplot of factor scores of the first two principal components.

altitudes (930, 1 634, 2 150 and 2 107 m elevation) the males do not differentiate between them, similar in size (with intermediate values) (Fig. 3). In the PC2 also showed the populations at higher and lower altitudes revealed bigger males, but 2 150 m elevation populations were significantly smalls (Post hoc test Bonferroni, $P < 0.001$).

Discriminant analysis generated three functions, the first highly significant ($P < 0.0001$) accounted for 55.19 % of the total variation. Although there was some overlap

among populations, when grouping all individuals 74.24 % were correctly assigned to their original population, and in general classification into incorrect was relatively low, except from populations of La Carolina (1 634 m.a.s.l.) (Table 3).

DISCUSSION

This study shows that: 1) the age differs significantly among the six populations of *Boana cordobae*, and as morphometric

TABLE 3
Discriminant function analysis. Percentage of correctly classified

| Function | 1 | Classification table | | | | | | | |
|-----------------------|--------|----------------------|----|------------|--------------|-------------|------------|-------------|------------|
| | | Actual group | n | 808 | 930 | 1634 | 2107 | 2150 | 2310 |
| Eigenvalue | 1.82 | 808 | 10 | 7 (70 %) | 0 (0.00 %) | 2 (20 %) | 0 (0.00 %) | 1 (10 %) | 0 (0.00 %) |
| Relative % | 55.19 | 930 | 26 | 0 (0.00 %) | 21 (80.77 %) | 3 (11.54 %) | 2 (7.69 %) | 0 (0.00 %) | 0 (0.00 %) |
| Canonical correlation | 0.80 | 1 634 | 11 | 1 (9.09 %) | 1 (9.09 %) | 5 (45.45 %) | 1 (9.09 %) | 2 (18.18 %) | 1 (9.09 %) |
| Wilk's lambda | 0.11 | 2 107 | 5 | 1 (20 %) | 0 (0.00 %) | 0 (0.00 %) | 4 (80 %) | 0 (0.00 %) | 0 (0.00 %) |
| χ^2 | 126.11 | 2 150 | 9 | 0 (0.00 %) | 0 (0.00 %) | 1 (11.11 %) | 0 (0.00 %) | 8 (88.89 %) | 0 (0.00 %) |
| df | 45 | 2 310 | 5 | 0 (0.00 %) | 0 (0.00 %) | 1 (20 %) | 0 (0.00 %) | 0 (0.00 %) | 4 (80 %) |
| P | 0.0000 | | | | | | | | |

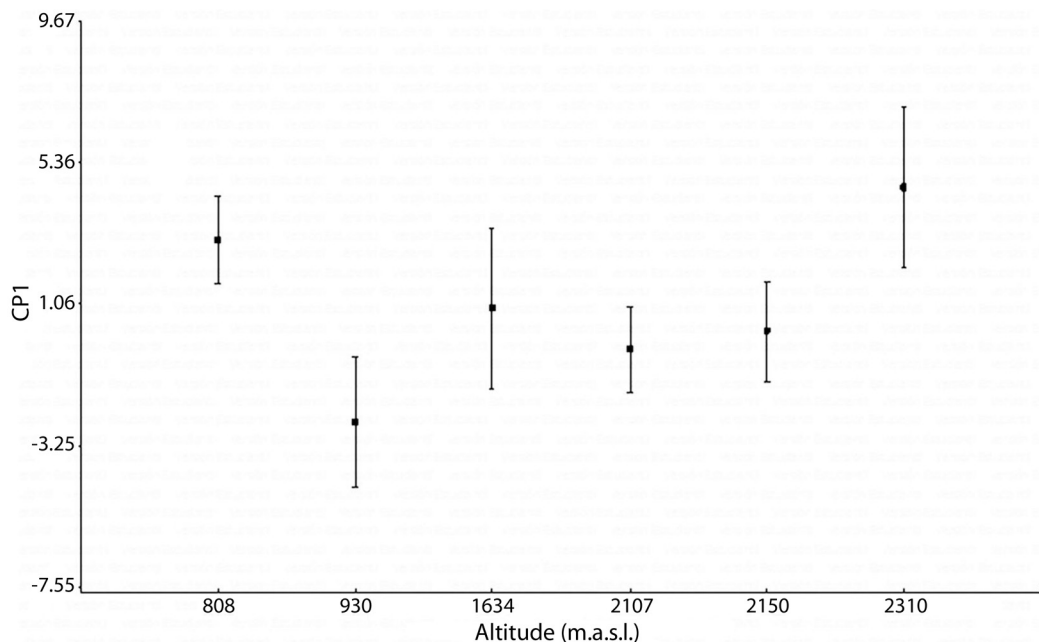


Fig. 3. Mean values with 95 % confidence intervals of PC1 for each populations (m.a.s.l.).

variables positively correlates to age, this age differences can partly explain the geographic variation of morphometric variables; 2) age-adjusted morphometric variables allow that differences among sites became more significant; 3) the patterns of variation in age and morphometric variables are related with the climate, and therefore this could be related to Bergmann's rule.

The presence of growth layers in bone tissue and counting the lines of arrested growth (LAGs) serve to evaluate age in *B. cordobae* (Otero et al., 2017a) and our study confirm that skeletochronology works well for age determination in the species.

Sexual size dimorphism is well known in most anurans (Mao et al., 2012; Liu et al., 2012; Lou et al., 2012; Otero et al., 2017b). In this study, there was a significant difference in most morphometric variables between sexes, even when the effect of age was removed, confirming the results of past studies on this species (Otero et al., 2017a). There is a marked sexual dimorphism; adult females are significantly larger than adult males. Numerous factors can explain sexual dimorphism in *B. cordobae* have been reported by these authors.

In our study, the relationship between age and several morphometric variables confirm similar results reported for this species (Otero et al., 2017a), where anurans exhibit indeterminate growth, implying that body size increases with age (Halliday & Verrell, 1988; Duellman & Trueb, 1994). Since body size can reflect individuals age in anurans (Liao & Lu, 2010; Ashkavandi et al., 2012; Altunişik and Özdemir, 2013; Otero et al. 2017a; Otero et al., 2017b) age is key as a covariate in morphometric studies.

Our results demonstrated that populations of *B. cordobae* vary in morphometric variables. We found a clinal trend in the morphometric variables: populations inhabiting different altitudes showed that individuals from higher altitudes were larger than those from lower altitude. In ectothermic animals with indeterminate growth, such as most amphibians, adult body size can show intra-specific geographic

variation, because associations between an organism's size and its environment (Angilletta & Dunham, 2003; Morrison & Hero, 2003; Rosso, Castellano, & Giacoma, 2004). It is expected that organisms exhibit larger adult size in colder environments (Partridge & French, 1996; Ashton, 2002; 2004; Lu et al., 2006), relation that even holds when altitude or latitude is used as a proxy for environmental temperature (Ashton, 2002; Angilletta & Dunham, 2003; Gül, Özdemir, Üzümlü, Olgun, & Kutrup, 2011; Altunişik and Özdemir, 2013). This fact is due cold temperatures retard both growth and development, and therefore individuals mature after and consequently the adults are larger and/or older (Morrison, Hero, & Browning, 2004; Rosso et al., 2004). As a result, high-altitude amphibians commonly are larger than those of low-altitude (Miaud et al., 1999; Iturra-Cid et al., 2010; Liao & Lu, 2010; Hsu, Hsieh, Hu, & Kam, 2014; Altunişik & Özdemir, 2015), the so-called Bergmann's rule.

Our data show that the highest values of morphometric variables and maximum life span were observed in the two populations with the higher altitude and the lower altitude, Los Linderos (2310 m elevation) and Achiras (808 m elevation). Furthermore, the ages (average age and longevity; Table 2) of specimens of these sites were higher than those from other populations. These results are noteworthy, because the morphometric variables and age are expected to be higher in highland populations as shown by other studies (Lai et al., 2005; Liao & Lu, 2010). Although part of our results confirms this trend, in this study, also average morphometric variables and age of the lowest population were higher. This fact shows that there are exceptions to the Bergmann's rule, with individuals in lowland population being significantly larger and older. Some authors associated these exceptions with parameters having effects on body size such as food availability, habitat quality, competition, and predation pressure on age at maturity, and limited altitudinal gradients (e.g.; Morrison & Hero, 2003). We believe that, individuals from Achiras were larger and older than in other

populations due the characteristics of the site where they live. Individuals were collected in water bodies associated to the river of a tourist locality, with presence of artificial light at night which favors the food availability and a longer growing season (personal communication). These same explanations were proposed by Liao et al. (2010b) when individuals of *Rana nigromaculata* from low altitudes were larger and older than those of higher sites.

In the results presented by Baraquet et al. (2012), individuals from Las Guindas (930 m elevation) showed lowest values in all morphometric variables and differing significantly from the other populations. However, in this paper age was not used as covariate. When age was included as a covariate, the posteriori test showed that the populations with higher differences were the sites with the higher altitude and the lower altitude, Los Linderos and Achiras. Thus, because body size may be a consequence of differences in age (Castellano & Giacoma, 2000), including individuals age improves significantly the results of studies evaluating morphometric variables (Kupfer, 2009).

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RESUMEN

Efecto de la edad en la variación geográfica de características morfológicas entre poblaciones de *Boana cordobae* (Anura: Hylidae). La variación morfológica del tamaño corporal es un aspecto importante de la historia natural de las especies, que ha sido de particular interés. En este estudio se estimaron diferencias en el tamaño corporal y la estructura de edad de seis poblaciones de *Boana cordobae* que viven a diferentes

altitudes, 808-2 310 m.a.s.l. en las provincias de Córdoba y San Luis (Argentina). La técnica de esqueletocronología se utilizó para la determinación de la edad, mediante la medición de 15 variables morfológicas en 79 individuos. Las variables morfológicas mostraron diferencias significativas entre sexos, siendo las hembras de mayor tamaño que los machos, incluso cuando el efecto de la edad se tuvo en cuenta; y se encontró una relación significativa entre la edad y la mayoría de las variables morfológicas. Cuando el efecto de la edad fue removido, se encontraron diferencias significativas en el tamaño del cuerpo entre las poblaciones estudiadas. Machos de poblaciones de mayor altitud son más grandes que los de altitudes menores. Estos resultados sugieren que las diferencias en la estructura de edad entre las poblaciones es un factor de importancia a tener en cuenta para analizar la variación en el tamaño corporal de esta especie según el área geográfica.

Palabras clave: *Boana cordobae*; esqueletocronología; variables morfológicas; variación geográfica.

REFERENCES

- Angilletta, M. J. & Dunham, A. E. (2003). The temperature-size rule in ectotherms: Simple evolutionary explanations may not be general. *The American Naturalist*, 162, 332-342.
- Angilletta, M. J., Steury, T. D., & Sears, M. W. (2004). Temperature, Growth Rate, and Body Size in Ectotherms: Fitting Pieces of a Life-History Puzzle. *Integrative and Comparative Biology*, 44, 498-509.
- Altunışık, A. & Özdemir, N. (2013). Body size and age structure of a highland population of *Hyla orientalis* Bedriaga, 1890, in northern Turkey (Anura: Hylidae). *Herpetozoa*, 26, 49-55.
- Altunışık, A. & Özdemir, N. (2015). Life history traits in *Bufo variabilis* (Pallas, 1769) from 2 different altitudes in Turkey. *Turkish Journal of Zoology*, 39, 153-159.
- Ashkavandi, S., Gharzi, A., & Abbassi, M. (2012). Age determination by skeletochronology (Anuran: Amphibia) in *Rana ridibunda*. *Asian Journal of Experimental Biological Sciences*, 3, 156-162.
- Ashton, K. G. (2002). Do amphibians follow Bergmann's rule? *Canadian Journal of Zoology*, 80, 708-716.
- Ashton, K. G. (2004). Sensitivity of intraspecific latitudinal clines of body size for tetrapods to sampling, latitude and body size. *Integrative and Comparative Biology*, 44, 403-412.
- Baraquet, M., Grenat, P. R., Salas, N. E., & Martino, A. L. (2012). Variación morfológica y geográfica entre poblaciones de *Hypsiboas cordobae* (Anura: Hylidae)



- en Argentina. *Cuadernos de Investigación UNED*, 4, 147-155.
- Baraquet, M., Salas, N. E., & Martino, A. L. (2013). Advertisement calls and interspecific variation in *Hypsiboas cordobae* and *Hypsiboas pulchellus* (Anura, Hylidae) from central Argentina. *Acta Zoologica Bulgarica*, 65, 79-486.
- Barrio, A. (1965). Las subespecies de *Hyla pulchella* Duméril & Bibron (Anura, Hylidae). *Phycis*, 69, 115-128.
- Bernal, M. H. & Clavijo, J. A. (2009). An essay on precision in morphometric measurements in anurans: inter-individual, intra-individual and temporal comparisons. *Zootaxa*, 2246, 32-44.
- Bionda, C., Kost, S., Salas, N., Lajmanovich, R., Sinsch, U., & Martino, A. (2015). Age structure, growth and longevity in the common toad, *Rhinella arenarum*, from Argentina. *Acta Herpetologica*, 10, 55-62.
- Castellano, S. & Giacoma, C. (2000). Morphometric and advertisement call geographic variation in polyploid green toads. *Biological Journal of the Linnean Society*, 70, 341-360.
- Cei, J. M. (1980). Amphibians of Argentina. *Monitore Zoologico Italiano, Italian Journal of Zoology*, 2, 1-609.
- Cvetković, D., Tomašević, N., Ficetola, G. F., Crnobrnja-Isailović, J., & Miaud, C. (2009). Bergmann's rule in amphibians: combining demographic and ecological parameters to explain body size variation among populations in the common toad *Bufo bufo*. *Journal of Zoological Systematics and Evolutionary Research*, 47, 171-180.
- De Queiroz, A. & Ashton, K. G. (2004). The phylogeny of a species-level tendency: species heritability and possible deep origins of Bergmann's rule in tetrapods. *Evolution*, 58, 1674-1684.
- Di Rienzo, J. A., Casanoves, F., Balzarini, M. G., Gonzalez, L., Tablada, M., & Robledo, C. W. (2018). *InfoStat versión*. Argentina: Grupo InfoStat, FCA, Universidad Nacional de Córdoba. Recuperado de <http://www.infostat.com.ar>
- Duellman, W. E. & Trueb, L. (1994). *Biology of amphibians*. McGraw-Hill, USA.
- Fattah, A., Slimani, T., El Mouden, E. H., Grolet, O., & Joly, P. (2014). Age structure of a population of *Barbarophryne brongersmai* (Hoogmoed 1972) (Anura, Bufonidae) inhabiting an arid environment in the Central Jbillets (West-Morocco). *Acta Herpetologica*, 9, 237-242.
- Green, D. E. (2018). *Anesthesia of amphibians in the field*. ARMI SOP No. 104: an online reference. Amphibian Research & Monitoring initiative National Wildlife Health Center 6006 Schroeder Road Madison.
- Retrieved from https://www.nwhc.usgs.gov/publications/amphibian_research_procedures/field_amphibian_anesthesia.pdf
- Gül, S., Özdemir, N., Üzümlü, N., Olgun, K., & Kutrup, B. (2011). Body size and age structure of *Pelophylax ridibundus* populations from two different altitudes in Turkey. *Amphibia-Reptilia*, 32, 287-292.
- Halliday, T. R. & Verrell, P. A. (1988). Body size and age in amphibians and reptiles. *Journal of Herpetology*, 22, 253-265.
- Heyer, W. R., Rand, A. S., Gonçalves da Cruz, C. A., Peixoto, O. L., & Nelson, C. E. (1990). Frogs of Boracéia. Frogs of Boracéia. *Arquivos de Zoologia. Museu de Zoologia da Universidade de São Paulo, (São Paulo)*, 31, 231-410.
- Hsu, F. H., Hsieh, Y. S., Hu, S. H., & Kam, Y. C. (2014). Altitudinal variation in body size and age structure of the Sauter's frog *Rana sauteri* in Taiwan. *Zoological Studies*, 53, 62.
- Iturra-Cid, M., Ortíz, J. M., & Ibarquengoytia, N. R. (2010). Age, Size, and Growth of the Chilean Frog *Pleurodema thaul* (Anura: Leiuperidae): Latitudinal and Altitudinal Effects. *Copeia*, 4, 609-617.
- Kupfer, A. (2009). Sexual size dimorphism in caecilian amphibians: analysis, review and directions for future research. *Zoology*, 112, 362-369.
- Lai, Y. C., Lee, T. H., & Kam, Y. C., (2005). A skeletochronological study on a subtropical, riparian ranid (*Rana swinhoana*) from different elevations in Taiwan. *Zoological Science*, 22, 653-658.
- Leclair, R., Leclair, M. H., Dubois, J., & Daoust, J. L. (2000). Age and size of wood frogs, *Rana sylvatica*, from Kuujuarapik, Northern Quebec. *The Canadian Field-Naturalist*, 114, 381-387.
- Lee, J. C. (1982). Accuracy and precision in anuran morphometrics: artifacts of preservation. *Systematic Zoology*, 31, 266-281.
- Li, S. T., Wu, X., Li, D. Y., Lous, S. L., Mi, Z. P., & Liao, W. B. (2013). Body size variation of odorless frogs (*Odorrana grahami*) across altitudinal gradients. *Herpetological Journal*, 23, 187-192.
- Liao, W. B. & Lu, X. (2010). Age structure and body size of the Chuanxi Tree Frog *Hyla annectans chuanxiensis* from two different elevations in Sichuan (China). *Zoologischer Anzeiger*, 248, 255-263.
- Liao, W. B., Lu, X., Shen, Y. W., & Hu, J. C. (2010a). Age structure and body size of two populations of the rice frog *Rana limnocharis* from different altitudes. *Italian Journal of Zoology*, 78, 215-221.
- Liao, W. B., Zhou, C. Q., Yang, Z. S., Hu, J. C., & Lu, X. (2010b). Age, size and growth in two populations of

- the dark-spotted frog *Rana nigromaculata* at different altitudes in southwestern China. *Herpetological Journal*, 20, 77-82.
- Liao, W. B. & Lu, X. (2011). Variation in body size, age and growth in the Omei Treefrog (*Rhacophorus omeimontis*) along an altitudinal gradient in western China. *Ethology Ecology and Evolution*, 23, 248-261.
- Liao, W. B., Lu, X., Shen, Y. W., & Hu, J. C. (2011). Age structure and body size of two populations of the rice frog *Rana limnocharis* from different altitudes. *Italian Journal of Zoology*, 78, 215-221.
- Liu, W. C., Liu, Y. H., Huang, Y., Mi, Z. P., & Li, C. (2012). Skeletochronological Study on Age Structure of a Chinese Endemic Frog (*Rana omeimontis*). *Asian Herpetological Reserch*, 3, 252-257.
- Lou, S. L., Jin, L., Liu, Y. H., & Mi, Z. P. (2012). Altitudinal variation in age and body size in Yunnan Pond Frog (*Pelophylax pleuraden*). *Zoological Science*, 29, 493-498.
- Lovich, J. E. & Gibbons, J. W. (1992). A review of techniques for quantifying sexual size dimorphism. *Growth, Development, and Aging*, 56, 269-281.
- Lu, X., Li, B., & Liang, J. J. (2006). Comparative demography of a temperate anuran, *Rana chensinensis*, along a relatively fine elevational gradient. *Canadian Journal of Zoology*, 84, 1789-1795.
- Ma, X. Y., Tong, L. N., & Lu, X. (2009). Variation of body size, age structure and growth of a temperate frog, *Rana chensinensis*, over an elevational gradient in northern China. *Amphibia-Reptilia*, 30, 111-117.
- Mao, M., Huang, Y., Mi, Z. P., Liu, Y. H., & Zhou, C. Q. (2012). Skeletochronological study of age, longevity and growth in a population of *Rana nigromaculata* (Amphibia: Anura) in Sichuan, China. *Asian Herpetological Reserch*, 3, 258-264.
- Martino, A. L. & Sinsch, U. (2002). Speciation by ploidy in *Odontophrynus americanus*. *Journal of Zoology*, 257, 67-81.
- Mi, Z. P. (2015). Age structure and body size in a breeding population of Asiatic toad (*Bufo gargarizans*) in southwestern China. *North-Western Journal of Zoology*, 11, 178-182.
- Miaud, C., Guyétant, R. & Elmberg, J. (1999). Variations in life-history traits in the common frog *Rana temporaria* (Amphibia: Anura): a literature review and new data from the French Alps. *Journal of Zoology*, 249, 61-73.
- Morrison, C. & Hero, J. M. (2003). Geographic variation in life-history characteristics of amphibians: a review. *Journal of Animal Ecology*, 72, 270-279.
- Morrison, C., Hero, J. M. & Browning, J. (2004). Altitudinal variation in the age at maturity, longevity, and reproductive lifespan anurans in subtropical Queensland. *Herpetologica*, 60, 34-44.
- Olalla-Tárraga, M. A. & Rodríguez, M. A. (2007). Energy and interspecific body size patterns of amphibian faunas in Europe and North America: anurans follow Bergmann's rule, urodeles its converse. *Global Ecology and Biogeography*, 16, 606-617.
- Otero, M., Baraquet, M., Pollo, F., Grenat, P., Salas, N., & Martino, A. (2017a). Sexual size dimorphism in relation to age and growth in *Hypsiboas cordobae* (Anura: Hylidae) from Córdoba, Argentina. *Herpetological Conservation and Biology*, 12, 141-148.
- Otero, M. A., Valetti, J. A., Bionda, C. L., Salas, N. E., & Martino, A. L. (2017b). Are ploidy and age size-related? A comparative study on tetraploid *Pleurodema kriegi* and octoploid *P. cordobae* (Anura: Leptodactylidae) from Central Argentina. *Zoologischer Anzeiger*, 268, 136 -142.
- Özdemir, N., Altunışık, A., Ergül, T., Gül, S., Tosunoğlu, M., Cadeddu, G., & Giacoma, C., (2012). Variation in body size and age structure among three Turkish populations of the treefrog *Hyla arborea*. *Amphibia-Reptilia*, 33, 25-35.
- Pancharatna, K. & Deshpande, S. A., (2003). Skeletochronological data on age, body size and mass in the Indian Cricket Frog *Limnonectes limnocharis* (BOIE, 1835) (Anura: Ranidae). *Herpetozoa*, 16, 41-50.
- Partridge, L. & French, V. (1996). Thermal evolution of ectotherm body size: Why get big in the cold? In I. A. Johnston & A. F. Bennett (Eds.), *Animals and temperature: Phenotypic and evolutionary adaptation* (pp. 265-292). Cambridge, UK: Cambridge University Press.
- Roff, D. A. (1992). *The Evolution of Life Histories: Theory and Analysis*. New York, USA: Chapman and Hall.
- Rosso, A., Castellano, S., & Giacoma, C. (2004). Ecogeographic analysis of morphological and life-history variation in the Italian treefrog. *Evolutionary Ecology*, 18, 303-321.
- Rozenblut, B. & Ogielska, M. (2005). Development and growth of long bones in Europea water frogs (Amphibia: Anura: Ranidae), with remarks on age determination. *Journal of Morphology*, 265, 304-317.
- Sinsch, U. (2015). Review: Skeletochronological assessment of demographic life-history traits in amphibians. *Herpetological Journal*, 25, 5-13.
- Todd, B. D., Scott, D. E., Pechmann, J. H., & Gibbons, J. W. (2011). Climate change correlates with rapid delays and advancements in reproductive timing in an amphibian community. *Proceedings of the Royal Society B: Biological Sciences*, 278, 2191-2197.