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AGE, BODY SIZE, AND GROWTH OF *BOANA CORDOBAE* (ANURA: HYLIDAE) ALONG AN ELEVATIONAL GRADIENT IN ARGENTINA

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***Abstract*.—Life-history traits of individuals organisms (e.g., longevity, age at sexual maturity, body size, and growth rate) often vary among populations in different environments, including along elevational gradients. We investigated elevational variation in the above variables for *Boana cordobae* using skeletochronology from 158 individuals in six populations along of an elevational gradient (930 to 2,310 m elevation) in central Argentina. Body size (snout-vent length) was the only variable that differed significantly between sexes, with females larger than males. Males were generally larger and older at higher elevations (females were not analyzed for populations differences due to small sample sizes). Specifically, body size was significantly positively correlated with elevation of the populations, with males from the highest elevation population significantly larger than those at lower elevations. Males at the highest site were also significantly older than individuals at lower elevation. Moreover, the oldest individual was 7 y old in the highest population, and 4–6 y old in the other populations, and age at sexual maturity was estimated at 3 y in the highest population and 2 y in the other five populations.**

Key Words.—geographic variation; life-history traits; skeletochronology

INTRODUCTION

Determining the age structure of amphibian populations is essential to determining several life-history traits (such as rate of growth, size at sexual maturity, and longevity) and to evaluating the status of populations by providing information on recent history, current state, and the future population trends (Dimmick and Pelton 1996; Ento and Matsui 2002; Yilmaz et al. 2005). Demographic analysis of populations has entailed the use of a variety of methods of age determination (Gibbons and McCarthy 1983). For amphibians skeletochronology is the most widely used technique for this purpose and it has been tested in numerous species (Sinsch et al. 2001; Sinsch 2015; Oromi et al. 2016). This method is based on the observation of annual growth rings in bones, commonly known as lines of arrested growth (LAGs), which are formed during the hibernation phase (Castanet et al. 1977). Formation of LAGs is genetically controlled based on a circannual rhythm (Castanet et al. 1993).

Life-history traits of amphibians often show variation among populations of the same species (Sagor et al. 1998; Miaud et al. 1999; Hsu et al. 2014; Altunışık and Özdemir 2015). Such variation is often attributed to differences in environmental variables (Lai et al. 2005; Özdemir et al. 2012), with temperature often exerting a strong effect (Liao et al. 2016). Consequently, elevation of the earth, as one common environmental gradient, mainly in temperature and season length, influences life histories of ectothermic organisms (Liu et al. 2012; Lou et al. 2012). This has been well documented in amphibian populations (Lu et al. 2006; Ma et al. 2009; Liao and Lu 2010; Huang et al. 2013). Populations at high elevations commonly have greater body size, delayed sexual maturity, greater longevity, and slower growth rate than populations at lower elevations (Iturra-Cid et al. 2010; Liao and Lu 2010; Hsu et al. 2014; Altunışık and Özdemir 2015).

Boana cordobae (Hylidae) is a frog endemic to Argentina. Its distribution is restricted to Córdoba and San Luis provinces, yet it inhabits a broad elevational range at 800–2,400 m (Baraquet et al. 2015). Although in recent years there have been numerous publications related to the species (e.g., Baraquet et al. 2014; Baraquet et al. 2015), there is a great lack of information on the demography of *B. cordobae*. Otero et al. (2017a) analyzed sexual size dimorphism in relation to age and growth, but the study was limited to a single population. Information about life-history traits across the species distribution is not available. Therefore, the aims of this study were to analyze age, body size, and growth of six populations of *B. cordobae* from different elevations to evaluate possible elevational effects on these life-history traits, and to compare demographic parameters between sexes and among populations.

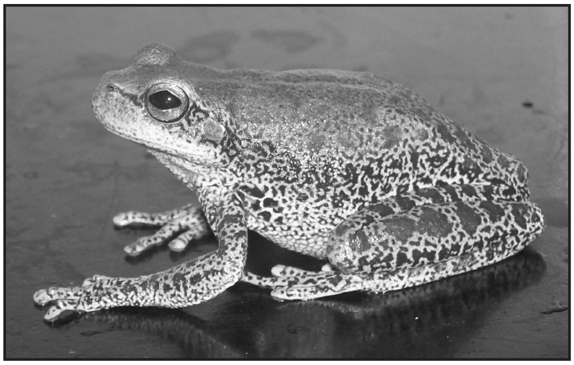

Figure 1. Adult male *Boana cordobae* from La Carolina (San Luis, Argentina). (Photographed by Mariana Baraquet).

MATERIALS AND METHODS

Study area and sampling.—We collected 158 individuals of *B. cordobae* (Fig. 1) between September 2006 and March 2014 (29 newly metamorphosed frogs, 104 adult males, and 25 adult females) from six populations of Cordoba and San Luis Provinces, Argentina, covering an area of about 20,000 km2 and an elevational range from 930 to 2,310 m: Las Guindas (32°35′35.22″S, 64°42′38.92″W, 930 m elevation), Cerro Aspero (32°50'34"S, 64°79'30"W, 1,200 m elevation), La Carolina (32°48′43.94″S, 66°05′48.15″W, 1,634 m elevation), Los Tabaquillos (32°23′59.75″S, 64°55′ 33.69″W, 2,107 m elevation), Pampa de Achala (31°49′41.8″S, 64°51′44.9″W, 2,150 m elevation), and Los Linderos (32°00′54.05″S, 64°56′42.97″W, 2,310 m elevation). We hand-captured adult individuals on the shores of streams and ponds and newly metamorphosed frogs while emerging from ponds. We determined the sex and the breeding condition of individuals using external secondary sexual characters (vocal sacs in males and eggs visible through the ventral skin of females). We measured the snout-vent length (SVL) of each individual using a digital caliper with a resolution of 0.01 mm. We clipped the longest right phalanx of each frog and preserved it in 70% ethanol.

Age determination.—We estimated age following standard skeletochronology methods (Castanet et al. 1993), cross sectioning the diaphysis at 10–12 µm using a rotary microtome, and staining with Ehrlich's haematoxylin (3 min). We identified and interpreted LAGs following Sinsch (2015) for neotropical species. Two researchers (Mariana Baraquet and Manuel Alejandro Otero) independently counted LAGs using a light microscope (Axiophot-Axiolab [100×]; Zeiss, Jena, Turingia, Germany) equipped with digital camera (G10, software Axio Vision 4.3; Canon, Tokyo, Japan). We identified double and false lines following Liao and Lu (2010) and assessed endosteal resorption based on the presence of the Kastschenko line (KL; the interface between the endosteal and periosteal zones; Rozenblut and Ogielska 2005). We also confirmed endosteal resorption following the protocol of Lai et al. (2005) comparing the major axis of the innermost LAG of each section with the mean of newly metamorphosed LAG without resorption. When the major axis of the innermost LAG of an adult was greater than the mean LAG of newly metamorphosed frogs without resorption, we assumed resorption had occurred.

Demographic variables and growth models.—We estimated demographic variables following Otero et al. (2017a,b): (1) age at sexual maturity: minimum number of LAGs counted in breeding individuals; (2) longevity: maximum number of LAGs counted in adults; (3) potential reproductive lifespan: difference between longevity and age at sexual maturity; (4) SVL at sexual maturity: average SVL of all first breeders with the minimum number of LAGs; and (5) modal lifespan: mode of age distribution. We constructed a growth model following the von Bertalanffy's (1938) growth equation, previously used in many studies of amphibians (e.g., Hsu et al. 2014; Liao et al. 2016; Otero et al. 2017a,b). We used the following equation: $SVL_t = SVL_{max} - (SVL_{max} - SVL_{met})\ e^{-K\,(t\,-\,tmet),}$ where SVL_t = average SVL at age t (mm); SVL_{max} = maximum asymptotic SVL (mm); SVL_{met} = average SVL at metamorphosis (mm); t = number of growing seasons experienced (age in years); t_{met} = proportion of the growing season until metamorphosis (age at metamorphosis fixed to 0.4 y according to Otero et al. 2017a); and *K* = growth coefficient.

Statistical analysis.—We used parametric tests when the data met the assumptions (normality and homogeneity of variance, tested with Shapiro-Wilks test) and non-parametric Mann-Whitney U-test to compare age between sexes, respectively. We computed Pearson's correlation coefficient to assess the relationship between body size and age in adults. We used analysis of covariance (ANCOVA) with age as the covariate to compare SVL between sexes. We also estimated the sexual size dimorphism (SDI): SDI = (size of larger sex / size of smaller sex) ± 1 (+1 if males were larger, −1 if females were larger), and the result arbitrarily defined as positive when females were larger than males and negative in the contrary (Lovich and Gibbons 1992).

For the analysis of geographic variation, we used only data from males because the number of captured females per population was low. Pearson's correlation coefficient was computed to assess the relationships between body size and elevation of the populations. We used ANOVA and Bonferroni post

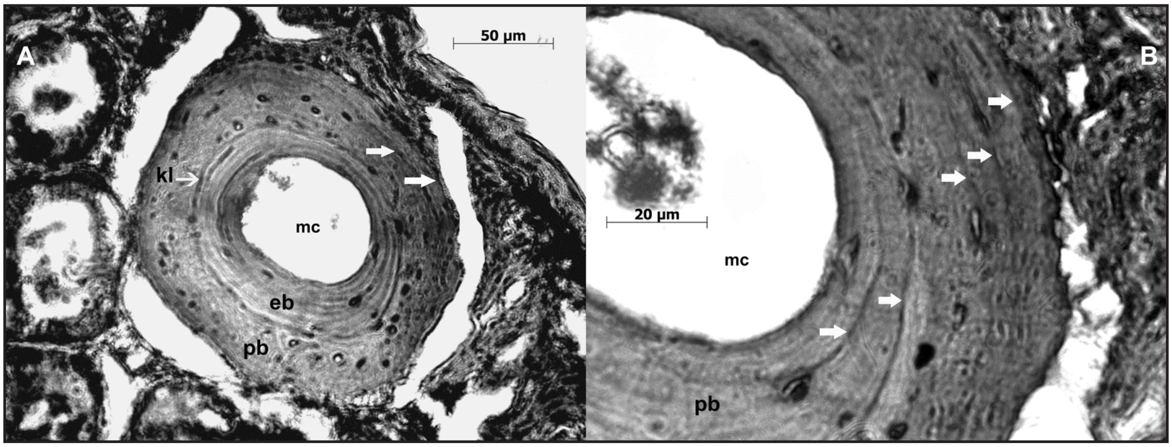

Figure 2. Examples of phalangeal cross-sections (12 μm thick) of *Boana cordobae*. (A) Male, snout-vent length (SVL) = 49.13 mm; 3 y old; the innermost line of arrested growth (LAG) has been eroded by endosteal resorption. (B) Male, SVL =54.87 mm; 5 y old. Wide white arrows = LAGs; mc= Medullar Cavity; pb = Periosteal Bone; eb= Endosteal Bone; kl (narrow white arrow) = Kastschenko Line. (Photographed by Mariana Baraquet).

hoc multiple comparisons for detection of significant differences among populations and we used an analysis of covariance (ANCOVA) with age as a covariate to compare body size among populations. For comparison of age among populations, we used Kruskal-Wallis test and Bonferroni post hoc multiple comparisons. We performed all tests in Statgraphics Centurion XVI.I, with $\alpha = 0.05$ considered significant.

Results

Skeletochronology.—All adult *B. cordobae* analyzed (104 males and 25 females) showed recognizable bone structures that allowed age determination. We found endosteal resorptions in 57% of the sample and observed well-defined Kastschenko lines (Fig. 2A), but the resorption did not hamper age determination because the first LAGs were never completely reabsorbed (Fig. 2B). We observed double and false LAGs, but we accounted for these anomalies and they did not affect LAGs counted in the sample.

Sexual variation in adult traits.—The SVL of females averaged 53.61 ± (SE) 5.26 mm (n = 25; range, 41.1–61.87 mm) and was significantly larger than SVL of males (n = 104; average 48.85 ± 3.32 mm; range, 41.0–61.23 mm; $F_{2,128} = 26.14$, $P < 0.001$). The sexual dimorphism index (SDI) in body size was 0.097, indicating an intersexual difference in body size with females larger than males. The SVL was significantly positively correlated with age in both sexes (males $r = 0.444$, $P < 0.001$; females $r = 0.417$, $P = 0.035$). When the effect of age was controlled (i.e., age was a covariable), there was a significant difference in body size between sexes ($F_{2,128} = 29.23$, $P < 0.001$). The SVL at sexual maturity was slightly higher in females (n = 5; average 50.60 ± 3.34 mm; range, 47–56.61) than in males (n = 19; average 47.01 ± 3.95 mm; range, 41.7–55.78), but the difference was not significant ($F_{2,128} = 2.21$, $P = 0.153$).

Demographic life-history traits of *B. cordobae* showed little variation between sexes (n = 104 males and 25 females). Mean age of breeding males was 3.32 ± (SE) 0.95 y (range, 2–7) whereas for females it was 3.44 ± 1.08 y (range, 2–7). There was no significant difference in age between sexes ($U = 1254.5$, $P = 0.831$). There were no sexual differences between sexes in minimum age at sexual maturity (2 y), longevity (7 y), potential reproductive lifespan (5 y), and modal age (3 y, 38.83% in males and 52% in females), although these variables could not be statistically tested.

Growth patterns.—Mean and standard deviation of SVL (SVL_{met}) for newly metamorphosed frogs was 27.57 ± 3.29 mm (range, 22.1–34.27 mm). The von Bertalanffy growth model fitted the age/body-length relationship well, with r = 0.92 and a percentage of variance explained (VE) of 86.16% in males and r = 0.95 and VE = 91.03% in females (Fig. 3). The estimated growth curve showed a similar shape in males and females. The growth coefficient *K* was higher in males (mean ± SE: 0.95 ± 0.12) than in females (0.76 ± 0.20), but this difference was not statistically significant because the 95% confidence interval of *K* for males (0.71–1.20) overlapped that for females (0.33–1.19). For both sexes, a decrease in growth rate was observed from the second to third year of age, just after the attainment of sexual maturity (i.e., 2 y in both sexes; Fig. 3). Although SVL_{max} did not differ significantly between sexes (males SVL_{max} = 50.70 mm, 95% confidence interval [CI] = 48.87–52.53; females SVL_{max} = 57.35 mm, CI = 52.40–62.30), the confidence intervals showed very little overlap, with females being larger than males.

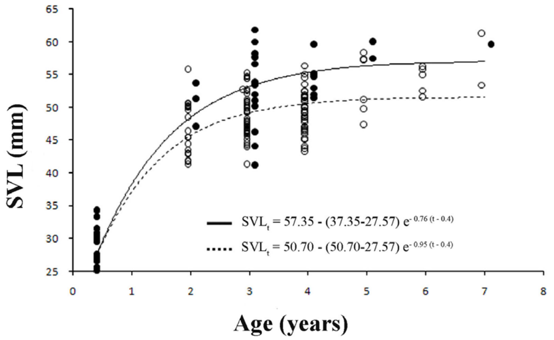

Figure 3. Relationship between snout-vent length (SVL) and age classes in *Boana cordobae*. Data are for 29 newly metamorphosed individuals (solid circles at 0.4 y age), 104 adult males (open circles), and 25 adult females (solid circles > 0.4 y age). Lines represent the von Bertalanffy's growth model (males: dotted line; females: solid line). Also shown are the von Bertalanffy's equations ($SVL_t = SVL_{max} - (SVL_{max} - SVL_{met})\ e^{-K\,(t - tmet)}$) with their respective values (see text for definition of terms). Sexual maturity was 2 y in both sexes.

Analysis of geographic variation.—For the analysis of geographic variation, we used only data from adult males (n = 104) because the number of captured females per population was low. SVL was correlated with elevation of each population ($r = 0.30$, $P = 0.002$) and SVL of males differed significantly among sites ($F_{6,103} = 7.37$, $P < 0.001$). Age had a significant effect on body size for males ($F_{6,103} = 16.93$, $P < 0.001$). When the effect of age was controlled, size differences in males among sites remained significant ($F_{6,103} = 5.83$, $P < 0.001$). Post-hoc comparisons for detection of differences among populations showed significant differences among the six populations (Bonferroni test, $P < 0.05$); males at the highest elevation (Los Linderos, 2,310 m) were significantly bigger than those at lower elevations (Table 1).

Age and elevation were not significantly correlated among the six populations ($P = 0.216$), and the average age did not differ significantly among populations ($F_{6,103} = 3.68$, $P = 0.615$). However, post hoc multiple comparisons indicated that the population at the highest elevation (Los Linderos, 2,310 m) differed significantly from the other populations (Bonferroni post hoc test, $P < 0.05$), with males averaging over 1 y older than males in the other populations. Moreover, the Los Linderos population showed greater longevity and delayed sexual maturity. Males at this site reached age 7 y whereas males in lower populations did not exceed 6 y old, and the minimum age at sexual maturity was 3 y at the Los Linderos site versus 2 y in all the others (Table 1). The von Bertalanffy growth model fitted the age/body length relationship in the six populations well, with r ranging among populations between 0.92–0.96 and percentage of variance explained between 89–93%. The SVL_{max} and growth coefficient (*K*) did not differ significantly among populations, because the 95% confidence intervals of SVL_{max} and *K* for all populations overlapped with each other (Table 1).

Discussion

Sexual size dimorphism has been observed in many amphibians, with females being larger than males (Liao and Lu 2011; Mao et al. 2012; Liu et al. 2012; Lou et al. 2012). Our study confirmed this pattern for *B. cordobae*. We also found a significant positive correlation between body size and age for both sexes, as has been reported in this and other species (Ashkavandi et al. 2012; Altunişik and Özdemir 2013; Otero et al. 2017a). Although the growth model variables, SVL_{max} and *K*, did not differ significantly between the sexes, the data strongly suggest that males reach a smaller asymptotic body

Table 1. Life-history traits of *Boana cordobae* from six populations of Cordoba and San Luis Provinces, Argentina, listed by elevation. Sample size (n) is listed for each elevation. Newly metamorphosed individuals (n = 29) were included in estimates of the von Bertalanffy's growth model. Mean (± standard error) are given for snout-vent length (SVL), age, SVL_{max}, and growth coefficient (*K*). The range of values is also given in parentheses for SVL_{max}, and *K*.

Variable	930 m (n = 26)	1,200 m (n = 33)	1,634 m (n = 12)	2,107 m (n = 5)	2,150 m (n = 22)	2,310 m (n = 5)
SVL (mm)	47.04 ± 2.51	48.45 ± 4.33	51.18 ± 3.23	49.59 ± 1.39	48.51 ± 3.08	56.09 ± 2.89
Age (y)	3.27 ± 0.83	3.21 ± 0.82	3.33 ± 1.15	3.00 ± 1.00	3.32 ± 0.78	4.60 ± 1.82
Longevity (y)	5	5	6	4	4	7
Age at sexual maturity (y)	2	2	2	2	2	3
Potential reproductive lifespan (y)	3	3	4	2	2	4
Modal age (%)	3 (38.46%)	3 (48.48%)	4 (33.33%)	4 (40%)	4 (50%)	3 (40%)
SVL_{max} (mm)	52.21 ± 3.14 (45.92–58.50)	61.99 ± 3.89 (54.22–69.77)	56 ± 3.48 (48.96–63.04)	51.70 ± 4.64 (42.25–61.15)	49.89 ± 2.67 (44.54–55.24)	58.29 ± 2.97 (52.24–64.35)
K	0.56 ± 0.17 (0.20–0.92)	0.33 ± 0.10 (0.12–0.53)	0.65 ± 0,23 (0.19–1.10)	0.94 ± 0.35 (0.22–1.65)	0.89 ± 0.22 (0.44–1.33)	0.75 ± 0.26 (0.22–1.27)

size (SVL_{max}) and have a greater growth coefficient (K) than females. If these differences are real, they could indicate that males reach their maximum length more rapidly than females, as observed in other amphibians (e.g., Li et al. 2010; Guarino et al. 2011) including *B. cordobae* (Otero et al. 2017a). Female anurans often mature at a larger size and greater age than males; this has been attributed to females needing more stored energy to allocate to gonad and embryo development (Monnet and Cherry 2002; Morrison et al. 2004).

Postmetamorphic growth in amphibians exhibits an indeterminate pattern with an exponential period that decreases considerably after the onset of reproduction as individuals approach asymptotic size (Hemelaar 1988; Yılmaz et al. 2005; Kyriakopoulou-Sklavounou et al. 2008; Guarino et al. 2011). *Boana cordobae* also demonstrated this pattern: growth rates for both sexes were high until sexual maturity, after which they decreased between the 2nd and 3rd year. We also observed that individuals of both sexes reached 95% of SVL_{max} at 4 y. This may have been because individuals used more energy for reproduction than for growth once they matured (Hemelaar 1988).

In contrast to the size-related traits above, other life-history traits evaluated showed little variation between sexes. No difference in maximum age was observed between sexes for *B. cordobae*, a result also reported for others anuran species (e.g., Friedl and Klump 1997; Kyriakopoulou-Sklavounou and Grumiro 2002). There was also no difference in age of sexual maturity between sexes. Given that some authors have suggested that age at sexual maturity is proportional to longevity (Houck 1982; Miaud et al. 2000), it would be expected that if there is no difference between the sexes in one of these variables there would also not be a difference for the other.

Several studies on amphibians have demonstrated intraspecific variation in life-history traits such as age and adult body size. In temperate regions, elevation, latitude, and the occurrence of cold periods are recognized as important variables in determining growth patterns (Caetano and Castanet 1993; Kyriakopoulou-Sklavounou and Grumiro 2002; Huang et al. 2013; Altunişik and Özdemir 2013). Numerous studies suggest that for amphibians, high-elevation populations produce larger and older individuals than their low-elevation counterparts (Miaud et al. 2000; Morrison and Hero 2003; Lu et al. 2006; Hsu et al. 2014; Altunışık and Özdemir 2015), and that age at sexual maturity and longevity are greater at high elevations (e.g., Morrison and Hero 2003; Zhang and Lu 2012; Hsu et al. 2014).

Our results showed that body size of *B. cordobae* males significantly increased with elevation, and individuals from the highest elevation (Los Linderos) were significantly larger than those from lower elevations. Age did not differ significantly among *B. cordobae* populations from different elevations, a finding consistent with some other anuran species (Liao et al. 2010; Gül et al. 2011). However, post hoc tests showed that individuals from the highest elevation population were significantly older than those in the other populations. Moreover, age at sexual maturity and maximum life span (longevity) were greatest in the highest elevation population, although it was not possible to conduct statistical tests for these data. Because anurans are often exposed to low temperatures at high elevations, the activity period is limited, and the growing season is restricted for high elevation populations. Thus, anurans at high elevations exhibit slower growth and developmental rates through metamorphosis and juvenile stages and, consequently, tend to live longer and mature later than lowland individuals (Miaud et al. 1999; Miaud et al. 2000; Morrison et al. 2004; Altunışık and Özdemir 2015). As a consequence of these effects, high-elevation amphibians commonly have a larger body size (Üzüm and Olgun 2009) and greater longevity (Morrison et al. 2004). This pattern even holds when elevation or latitude is used as an indicator for environmental temperature (Ashton 2002).

In conclusion, our life-history traits assessment allowed us to determine sexual size dimorphism, with females larger than males. Moreover, we observed elevational variation in mean body size, age, age at sexual maturity, longevity, and growth among six populations of *B. cordobae*, and we suggest that differences in temperature are the reason for the variations in these life-history traits. Also, our results show the importance of age determination for morphometric analysis and it geographic variation.

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