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Research paper

## Are ploidy and age size-related? A comparative study on tetraploid *Pleurodema kriegi* and octoploid *P. cordobae* (Anura: Leptodactylidae) from Central Argentina

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### ABSTRACT

Size differences of anuran amphibian complexes with different ploidy levels are well known, whereas the larger species usually corresponds to a lower ploidy level. Neotropical *Pleurodema kriegi* (tetraploid) and *P. cordobae* (octoploid) differ in many morphometric variables and, in all cases, the species with the higher ploidy level corresponded to an increased body size. Although sexual size dimorphism (SSD) has been tested in *P. kriegi* without considering age-related differences in body size, SSD in *P. cordobae* is unknown. Here we determined individual age in order to analyse: (1) the intraspecific pattern of SSD of both species and (2) whether a larger body size and lower ploidy level relation observed in other polyploid anuran amphibians are reversed in a tetra–octoploid complex. Age and body size (snout–vent length) were correlated in both sexes and species. When the effect of age was controlled, significant a SSD were found. Intraspecific differences in demographic parameters were significantly higher than interspecific ones. We confirmed a female-biased SSD for both species and found the first case in which the larger species corresponds to a higher ploidy level. Different ploidy levels were an important factor determining age and body size under similar environmental conditions. Skeletochronology provided basic demographic data of *P. kriegi* and *P. cordobae* which are now available for comparative and conservation studies.

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### 1. Introduction

Polyploidy is an important evolutionary force in some plant groups, (White, 1973; Otto and Whitton, 2000) and tend to have overall increased size when compared with the diploids. However the mechanism is also reported in insects, crustaceans, molluscs, fish, amphibians, reptiles and (to a lesser extent) mammals (reviewed in Bogart, 1980; Lewis, 1980; Otto and Whitton, 2000; Le Comber and Smith, 2004; Gregory and Mable, 2005; Schmid et al., 2010; Evans et al., 2012). Among anuran amphibians polyploidy has been documented in several families (Kawamura, 1984; Kuramoto, 1990; Mable et al., 2011; Bogart and Bi, 2013; Schmid et al., 2015).

There are well-studied examples of anuran amphibians complexes with variable ploidy levels that differ in size e.g., *Hyla*

*crhysoscelis* (2×)/*H. versicolor* (4×) (Holloway et al., 2006); *Bufo viridis* complex 2×/4× (Stöck, 1998) and *Odontophrynus cordobae* (2×)/*O. americanus* (4×) (Martino and Sinsch, 2002; Grenat et al., 2012). In all these cases the larger species corresponded to a lower ploidy level. Polyploid amphibians probably maintain higher cell volumes because of an increased DNA-content but hold reduced total cell numbers compared to diploid congeners (Bogart, 1980). However, Valetti et al. (2009) reported the reversed relationship in neotropical *Pleurodema kriegi* (tetraploid, 4×) and *P. cordobae* (octoploid, 8×) as in ten out of sixteen morphometric variables the species with higher ploidy level showed a larger body size. However, the lack of information on the age of individuals analyzed prevented determining whether this result was due to an interspecific size difference *per se* or if it was associated with different age structures of the population. Furthermore, to carry out morphological comparison between species more accurately, is essential to determine if there is sexual size dimorphism (SSD). This is a widespread phenomenon in numerous anuran species in which either females (Bosch and Márquez, 1996 Reinhard et al., 2015) or

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males (Taborsky et al., 2009) have a larger body size. Duellman and Veloso (1977) noted a female-biased SSD in *P. kriegi* as well in other *Pleurodema* species without discussing age relations, whereas SSD in *P. cordobae* has not been studied. Consequently, SSD considering age related differences must be assessed in both species. Moreover, ageing is also important to assess the growth rate, age and size at sexual maturity and the species longevity.

The aim of the present study was to assess the individual age and demographics parameters of tetraploid *P. kriegi* and octoploid *P. cordobae* populations in order to test two exclusive hypotheses; H1: both species have a female-biased SSD; and H2: A larger body size and lower ploidy level relation observed in other amphibian ploidy complexes is reversed in the tetra-octoploid complex of *Pleurodema*.

## 2. Materials and methods

### 2.1. Study species

The neotropical frog genus *Pleurodema* Tschudi, 1838 (Anura: Leptodactylidae) is represented by fifteen species (Frost, 2016), three of which are polyploid. *Pleurodema kriegi* and *P. bibroni* are both tetraploid, whereas *P. cordobae* is octoploid (Valetti et al., 2009). *Pleurodema kriegi* and *P. cordobae* are cryptic endemics of the Sierra Grande de Córdoba, whereas *P. bibroni* is restricted to Uruguay up to the State of Rio Grande do Sul in Brazil (Barrio, 1977; Kolenc et al., 2009). *Pleurodema kriegi* has a narrow distribution in the northern Pampa de Achala, while its sister species *P. cordobae* has been reported further south (Pampa de Achala to the Sierra de Comechingones, see Valetti et al., 2013). The individual assignment to the species *P. cordobae* or *P. kriegi* was performed through ploidy level. Ploidy of the individuals was determined by cytogenetic analysis following the technique used by Baraquet et al. (2011) and estimated by comparing erythrocyte nuclei size of adult individuals following Otero et al. (2013). For the study we used samples of preserved *Pleurodema* specimens kept at Herpetological Collection (GIHRC), Department of Natural Sciences, Faculty of Exact, Physical-Chemical and Natural Sciences, National University of Río Cuarto. The vouchers were collected in 2013 at two different locations with similar climatic conditions in Sierra Grande de Córdoba: 49 individuals of *P. kriegi* (4 froglets, 4 juveniles, 30 adult males and 11 adult females) from La Posta (31°36'46"S, 64°52'29"W, 2151 m a.s.l.) and 57 individuals of *P. cordobae* (5 froglets, 2 juveniles, 31 adult males and 19 adult females) from Los Tabaquillos (32°23'58"S, 64°55'35"W, 2105 m a.s.l.). Specimens analyzed are listed in the Appendix A.

### 2.2. Capture, measurements, and sex determination

All specimens were captured by hand at the beginning of the breeding season (September–December). Newly metamorphosed froglets tails were not completely reabsorbed and were captured emerging from ponds. Juveniles showed adult body characteristics and were captured by an active search under rocks. Adult males were captured emitting their advertisement call and adult females were found in amplexus. Adult frogs were sexed according to external secondary sexual characteristics (black vocal sacs in males, readily visible eggs through the ventral skin of females) and the snout-vent length (SVL to the nearest 0.01 mm) was measured by a digital caliper. The longest right phalanx of each frog was clipped off and preserved in a 70% solution of alcohol.

### 2.3. Age determination

Laboratory protocols for age assessment were performed following the standard methods of skeletochronology (e.g. Martino

and Sinsch, 2002; Liao et al., 2010; Bionda et al., 2015; Sinsch, 2015). The diaphyseal portion of each phalanx was cross-sectioned at a thickness of 8–10  $\mu\text{m}$  by a rotary microtome Leica® RM2125 RTS. Histological sections were stained for 3 min in Ehrlich's hematoxylin and rinsed with distilled water. Periosteal lines of arrested growth (LAGs), corresponding to each year of life 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. Double and false lines (incomplete and faded hematoxylinophilic lines) were defined following the suggestion of Sinsch et al. (2007). Endosteal resorption of long bones started from the inner surface of the bone, enlarging the marrow cavities and eroding a portion of LAGs when frogs have completed their hibernation (Rozenblut and Ogielska, 2005). Resorption was confirmed by observing the presence of the Kastschenko Line (KL; the interface between the endosteal and periosteal zones; Rozenblut and Ogielska 2005). The complete resorption of the innermost LAG was also confirmed by the difference in LAGs and KL diameter (sensu Liao and Lu, 2010a; Li et al., 2013).

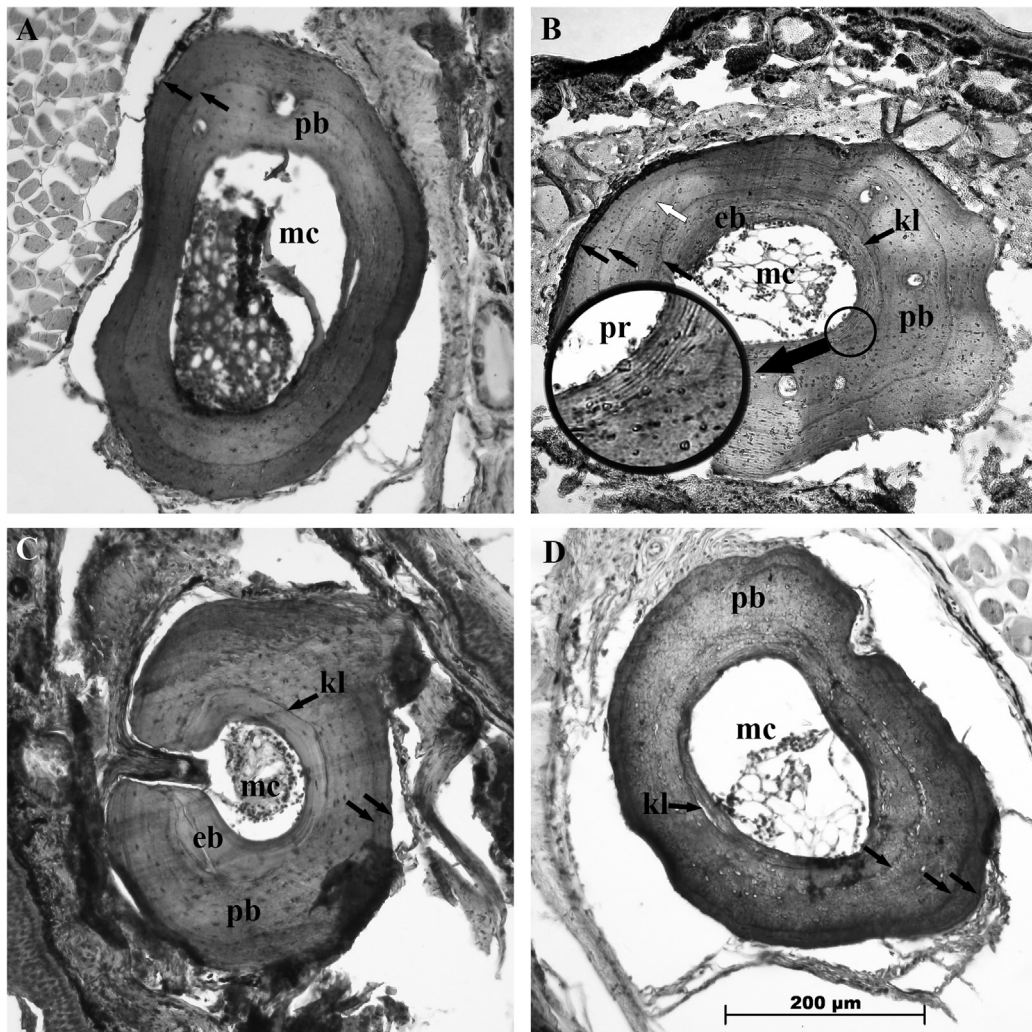
### 2.4. Demographic parameters and growth models

The following demographic variables were calculated according to Bionda et al. (2015): (1) age at maturity: the minimum number of LAGs counted in breeding individuals; (2) longevity: the maximum number of LAGs counted in mature frogs; (3) potential reproductive periods: the difference between longevity and age at maturity; (4) size at maturity: the average snout-vent length of all first breeders with the minimum number of LAGs; (5) modal lifespan: mode of age distribution.

Growth was estimated according to von Bertalanffy's (1938) equation, previously used in several studies of amphibians (e.g., Cogălniceanu and Miaud, 2002; Miaud et al., 2007; Üzümlü and Olgun, 2009; Guarino et al., 2011; Liao and Lu, 2012; Marangoni et al., 2012; Bionda et al., 2015). We used the following equation:  $SVL_t = SVL_{max} - (SVL_{max} - SVL_{met})e^{-K(t - t_{met})}$ , where  $SVL_t$  = average SVL at age  $t$ ,  $SVL_{max}$  = maximum asymptotic SVL,  $SVL_{met}$  = average SVL at metamorphosis,  $t$  = number of growing season experienced (age),  $t_{met}$  = proportion of the growing season until metamorphosis (age at metamorphosis, fixed to 0.25), and  $K$  = growth coefficient (shape of the growth curve). The von Bertalanffy growth model was fitted to the average growth curve using the least square procedure and the growth model of body length was regarded as a function of age.

### 2.5. Statistic analysis

Regression analyses were performed to determinate relationships between body size (SVL) and age in adults of both species. All characters measured were tested for normality using Shapiro-Wilks test. Since (1) the groups (sex and species) were heterogeneous in age, and (2) the body size was positively correlated with age, analyses of covariance (ANCOVAs) using age as a covariate were carried out to compare SVL between sexes (intraspecific) and SVL between species of the same sex (interspecifically). We also estimate the sexual dimorphism index (SDI) according to Lovich and Gibbons (1992).  $SDI = (\text{size of larger sex} / \text{size of smaller sex}) \pm 1$ . +1 if males are larger or -1 if females are larger, and arbitrarily defined as positive when females are larger than males and negative in the contrary. A probability level of  $P < 0.05$  was considered significant. All tests were performed with the Statistica 5.0/W software package (Statsoft Inc., USA).



**Fig. 1.** Examples of phalangeal cross-sections of the *Pleurodema* complex. (A) Males of *P. kriegi*; SVL: 32.1 mm; 3- year-old; the innermost LAG has been eroded by endosteal resorption. (B) Female of *P. kriegi*; SVL: 40.41 mm; 3- year-old; the second LAG has been considered as false. (C) Male of *P. cordobae*; SVL: 33.97 mm; 2-year-old. (D) Female of *P. cordobae*; SVL: 39.94 mm; 3-year-old. Black Arrows = lines of arrested growth (LAGs); White Arrows = False LAGs; mc = Medullary Cavity; pb = Periosteal Bone; eb = Endosteal Bone; kl = Kastschenko Line. (B) See zoom of cross-section showing the Partial Resorption (pr) of innermost LAG.

### 3. Results

#### 3.1. Skeletochronology

The hematoxylin-stained cross sections of phalanges (Fig. 1) showed a series of narrow concentric hematoxylinophilic rings (LAGs) separated by wide growth layers and in some cases showed the presence of the Kastschenko line. The outer bone perimeter was counted as a LAG because all frogs were collected at the beginning of the breeding season. In all of the studied specimens the resting lines were distinct and easily counted (Fig. 1). Endosteal resorption was present in 19% of the sample and in six individuals the innermost LAGs was completely reabsorbed (e.g., Fig. 1A). False LAGs were observed in two individuals (see Fig. 1B), whereas double LAGs were not observed in any sample.

#### 3.2. Body length and age

Demographic life-history traits of *Pleurodema* complex varied substantially among species and between sexes (see Table 1). SVL was significantly correlated with age on both sexes in *P. kriegi* (Males,  $r=0.58$ ,  $P\leq 0.01$ ; Females,  $r=0.48$ ,  $P=0.019$ ) and *P. cordobae* (Males,  $r=0.62$ ,  $P\leq 0.01$ ; Females,  $r=0.46$ ,  $P\leq 0.01$ ), showing

greater SVL with increasing age (Table 2, Fig. 2). The ANCOVA, controlled for age, revealed significant sexual difference in body size within species (*P. kriegi*,  $F_{1,38} = 45.22$ ,  $P < 0.01$ ; *P. cordobae*  $F_{1,46} = 81.71$ ,  $P < 0.01$ ) with females being larger than males. Inter-specific size differences of both sexes were observed, where *P. cordobae* was larger than *P. kriegi* (Males,  $F_{1,57} = 45.17$ ,  $P < 0.01$ ; Females,  $F_{1,27} = 30.23$ ,  $P = 0.015$ ). The sexual dimorphism index (SDI) in body size was 0.097 for *P. kriegi* and 0.133 for *P. cordobae* (implying tendency towards a female-biased dimorphism in both species).

#### 3.3. Growth patterns

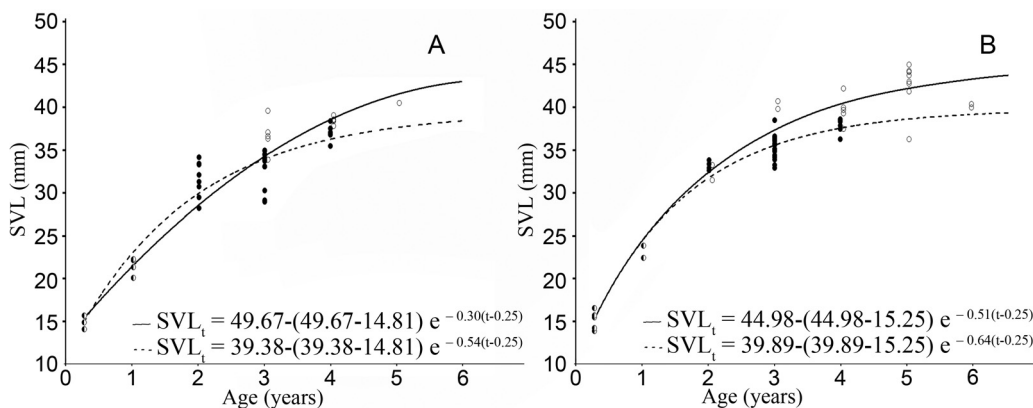
Mean and standard deviation of recently metamorphosed froglets SVL (SVL<sub>met</sub>) was  $14.81 \pm 0.81$  mm in *P. kriegi* and  $15.24 \pm 1.11$  mm in *P. cordobae*. The von Bertalanffy (1938) growth model correctly fitted the age/body-length relationship (Fig. 2). The estimated growth curve showed a similar shape in males and females but the growth coefficient was higher in males than in females in both species (Table 3). The maximum asymptotic SVL<sub>max</sub> differed between sexes in *P. kriegi* (Male SVL<sub>max</sub> = 39.38 mm, CI95%: 35.28–41.50; Females SVL<sub>max</sub> = 49.67 mm, CI95%: 42.18–59.23), while in *P. cordobae* the 95% confidence intervals of SVL<sub>max</sub>

**Table 1**  
 Demographic life history traits of adult Males (♂) and adult Females (♀) of tetraploid (4n) *P. kriegi* and octoploid (8n) *P. cordobae*. Mean ± SE and ranges (in parentheses) are given.

Specie	Sex	N	Adult SVL mean	Agemean (Years)	Age Mode (frequency)	Age at sexual maturity (Years)	Longevity (Years)	Potential reproductive lifespan (Years)
<i>P. k</i> (4n)	♂	30	34.41 ± 2.6 (28.13–38.31)	2.93 ± 0.69 (2–4)	3 (53.3%)	2	4	3
	♀	11	37.76 ± 1.81 (33.81–40.41)	3.63 ± 0.67 (3–5)	4 (45.5%)	3	5	3
<i>P. c</i> (8n)	♂	31	35.69 ± 1.74 (32.74–38.73)	3.06 ± 0.57 (2–4)	3 (67.7%)	2	4	3
	♀	19	40.43 ± 3.63 (31.62–45.14)	4.31 ± 1.15 (2–6)	5 (36.8%)	2	6	5

**Table 2**  
 Snout-vent length (SVL) for age class of individuals of *P. kriegi* and *P. cordobae*. Sample size (bold italic), mean ± SE and ranges (in parentheses) are given.

Species	Sex	Age (Years)							
		0.25	1	2	3	4	5	6	
<i>P. kriegi</i> (4n)	Male	<b>3</b> 14.81 ± 0.81 (14–15.62)	<b>4</b> 21.38 ± 0.99 (20.02–22.19)	<b>8</b> 31.50 ± 2.057 (28.13–34.05)	<b>16</b> 33.05 ± 1.94 (28.89–34.88)	<b>6</b> 36.94 ± 0.95 (35.4–38.31)	–	–	–
	Female	–	–	–	<b>5</b> 36.61 ± 2.02 (33.81–39.49)	<b>5</b> 38.38 ± 0.45 (37.74–39)	<b>1</b> 40.41	–	–
<i>P. cordobae</i> (8n)	Male	<b>5</b> 15.24 ± 1.1 (13.96–16.62)	<b>2</b> 23.22 ± 1.02 (22.5–33.39)	<b>4</b> 33.32 ± 0.54 (32.74–33.97)	<b>21</b> 35.49 ± 1.26 (33.03–38.63)	<b>6</b> 37.9 ± 0.87 (36.4–38.73)	–	–	–
	Female	–	–	<b>2</b> 32.51 ± 1.25 (31.62–33.39)	<b>2</b> 40.42 ± 0.67 (39.9–40.89)	<b>5</b> 39.88 ± 1.68 (37.62–42.34)	<b>8</b> 42.78 ± 2.75 (36.41–45.14)	<b>2</b> 40.33 ± 0.26 (40.15–40.52)	–



**Fig. 2.** Relationship between body length (SVL) and age classes in *Pleuroderma kriegi* (A) and *P. cordobae* (B). Lines represent the von Bertalanffy growth model (Males: Solid line; Female: dotted line). Also shown the Von Bertalanffy equations ( $SVL_t = SVL_{max} - (SVL_{max} - SVL_{met}) e^{-K(t-t_{met})}$ ) with their respective values.

**Table 3**  
 Growth parameters (mean ± SE) of both sex in *P. kriegi* and *P. cordobae*, estimated by von Bertalanffy's growth model. Additionally, for all estimates were included the froglets and juveniles to each species.

Sample	<i>P. kriegi</i> (4n)		<i>P. cordobae</i> (8n)	
	Males	Females	Males	Females
Sample	37	18	38	26
Asymptotic size (SVL <sub>max</sub> in mm)(CI <sub>95%</sub> )	39.38 ± 1.39(35.28–41.50)	49.67 ± 4.02(42.18–59.23)	39.89 ± 1.07(37.7–42.06)	44.98 ± 1.57(41.16–47.23)
Growth coefficients (K) (CI <sub>95%</sub> )	0.54 ± 0.084 (0.38–0.70)	0.30 ± 0.05 (0.17–0.42)	0.64 ± 0.08 (0.49–0.80)	0.51 ± 0.08 (0.37–0.66)
R	0.952	0.988	0.986	0.981
Variance Explained (r <sup>2</sup> )	91.32%	97.71%	97.28%	96.23%

overlapped (Male SVL<sub>max</sub> = 39.89 mm, CI95%: 37.7–42.06; Females SVL<sub>max</sub> = 44.98 mm, CI95%: 41.16–47.23). Males had a smaller asymptotic size but a greater growth coefficient (K) than females in both species (Table 3); indicating that males reach their maximum length more rapidly than females. Although females were

larger than males throughout their life, each age class of both sexes showed great variability. K for males were slightly higher in *P. cordobae* than in *P. kriegi* (0.38–0.70 for *P. kriegi* and 0.49–0.80 for *P. cordobae*) and in females (0.17–0.42 for *P. kriegi* and 0.37–0.66 for *P. cordobae*), however these differences were not statistically signifi-

**Table 4**The Annual Growth Rates (R) of both sex in *Pleurodema kriegi* and *Pleurodema cordobae* for all Age class.

Age (year)	R (mm/year)			
	<i>P. kriegi</i> (4n)		<i>P. cordobae</i> (8n)	
	Males	Females	Males	Females
1	9.71	8.49	10.67	11.01
2	4.26	–	4.21	6.36
3	3.42	3.92	2.81	2.33
4	1.32	3.39	1.22	2.6
5	–	2.78	–	2.31
6	–	–	–	1.12

cant because the 95% confidence intervals of K overlapped in both species. Annual growth rates (R) decreased markedly in both sexes after maturity. R of juveniles of *P. cordobae* were higher than in *P. kriegi*, while in adults R of *P. kriegi* were higher than in *P. cordobae* (Table 4).

#### 4. Discussion

Intersexual comparison of body size controlled for age between both species, confirmed a female-biased SSD. We confirmed that octoploid frogs were larger than tetraploid and thus presented the first case in which a larger species corresponded to a higher ploidy level among polyploid anuran complexes.

Age determination through LAGs counting was essential to corroborate our hypothesis. LAGs formation is considered to be mainly genetically controlled underlying a circannual rhythm (e.g., Bionda et al., 2015; Sinsch, 2015). Several studies in palearctic and temperate anurans confirmed the formation of one LAG per year, equivalent to the number of hibernations (e.g., Marangoni et al., 2012; Bionda et al., 2015). Also in *P. kriegi* and *P. cordobae* the formation of growth layers may be associated with a marked seasonality resembling a temperate regime with high-elevation habitats. Although some authors have demonstrated that bone resorption never, or only partially, reached to the innermost LAGs (Gibbons and McCarthy, 1983; Sinsch et al., 2001; Iturra-Cid et al., 2010; Sinsch et al., 2001; Iturra-Cid et al., 2010), others studies have shown that endosteal resorption can erode one or more LAGs (e.g., Tsiora and Kyriakopoulou-Sklavounou, 2002; Guarino et al., 2008; Cogălniceanu and Miaud, 2003; Miaud et al., 2007) consistent with our observations of *P. kriegi* and *P. cordobae*. Moreover, double and false LAGs may lead to an over estimation of age (Sinsch et al., 2007; Sinsch, 2015). False LAGs were also observed in agreement with previous studies (Li et al., 2010, 2013). The identification of bone resorption in some phalange cross sections, and the detection of false LAGs in other ones, allowed us to estimate the age of individuals more accurately.

##### 4.1. Sexual size dimorphism

Adult females of both *P. kriegi* and *P. cordobae* were significantly larger than males. Natural selection should favour large females when female size is positively correlated with fecundity (Gibbons and McCarthy, 1986; Shine, 1988; Liao et al., 2015) and a general positive correlation between body length and fecundity is well-documented among anurans (e.g., Davies and Halliday, 1977; Kaplan and Salthé, 1979; Liao et al., 2015). In *P. kriegi* and *P. cordobae*, SVL did not significantly differ between sexes at sexual maturity, although the SVL in older age classes was significantly different between sexes, with females being largest. Therefore, greater female length is unlikely to be linked to pre reproductive age nor to delayed sexual maturity in females, both patterns seen in several species of frogs (Miaud et al., 1999; Eaton et al., 2005; Liao and Lu, 2010b, 2012; Bionda et al., 2015). It appears that females of *P. kriegi*

reach sexual maturity a year later than males as reported for many amphibians (e.g., Patón et al., 1991; Miaud et al., 1999; Bastien and Leclair, 1992; Eaton et al., 2005; Bionda et al., 2015). However, the lack of two-year old females in our sample does imply that *P. kriegi* females cannot mature at two years.

Both in *P. kriegi* and *P. cordobae*, sexual size differentiation is not reached with sexual maturity ad hoc but differentiation occurs in older ages, when more resources are allocated to reproduction (Halliday and Verrell, 1988). Our von Bertalanffy growth models showed that there are few differences in growth rates of immature *P. kriegi* and *P. cordobae* whereas the growth curves distinctly diverge between sexes from three years onwards. Anuran age structure of both sexes may vary markedly between the years in the same population (Driscoll, 1999). As our data were based on a single year, long-term studies are needed for a better understanding of the dynamics of age structure in populations of *P. kriegi* and *P. cordobae*.

##### 4.2. Interspecific comparisons

The role of polyploidization as a creative force in producing evolutionary novelty will be mediated through its effects on the phenotype (Otto and Witon, 2000). Polyploidy is sometimes, associated with larger overall size in plant (Stebbins, 1950) and invertebrates (Bell, 1982). In fishes and amphibians polyploidy tends to have little to no effect on body size (Fankhauser, 1945; Mahony and Robinson, 1980; Schultz, 1980). The results of this study indicate that the age structure of males would not differ between species. The lack of two-year-old *P. kriegi* females in the sample but the presence of six year-old *P. cordobae* females generated some uncertainty about the demography in natural populations. Here, comparative analysis of SVLs considering age showed that males of *P. cordobae* are larger than males of *P. kriegi*. This could give an important indication that the previously reported difference in size between these two species (Valetti et al., 2009) are not linked to differences in age structure but rather individuals of *P. cordobae* reach sexual maturity at larger size than *P. kriegi* (see also a higher growth coefficient –K– and the highest growth rate –R– for juveniles) and then maintain a similar growth pattern during adult hood. Developmental rates have been shown to be altered in polyploid plants, amphibians, and insects, with polyploids generally having slower developmental rates than related diploids (e.g. Fankhauser, 1945; Lowcock, 1994; von Well and Fossey, 1998). Martino and Sinsch (2002), using individual age, show the same relation in diplo-polyploid complex *Odontophrynus cordobae* (2x)/*O. americanus* (4x). However, our results show a reversed relationship since the lower ploidy species have slower developmental rates. It is worth noting that demographic parameters analyzed depend heavily on environmental variables such as temperature and altitude in which populations are located (e.g., Liao and Lu, 2010a,b; Iturra-Cid et al., 2010; Bionda et al., 2015; Sinsch et al., 2015). The localities of the two study species were geographically relatively close (ca. 90 km), shared similar altitudes and

seasonal temperature regimes and also the breeding sites had similar characteristics (Valetti et al., 2009, 2013). The genetic factor, under similar environmental conditions, may bring about inter- and intra-population differences in age and body size (Berven, 1982; Miaud et al., 1999). Therefore, to minimize environmental variance on this analysis, we could show that the differences found between polyploid *P. kriegi* and *P. cordobae* would have a large genetic component.

Differences in demographic parameters were significantly higher between sexes than between species. Our results showed that differences in the ploidy level could be an important factor in determining age and body size. In conclusion, age assessment and subsequent growth modeling allowed us to determine female-biased SSD for (tetra/octoploid) *P. kriegi*/*P. cordobae*. Moreover, we verified that within the *Pleuroderma* complex, octoploid *P. cordobae* have a larger body size. Our basic demographic data from natural populations of *P. kriegi* and *P. cordobae* are now available for future comparative and conservation studies.

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### Appendix A. : Specimens analyzed

#### *Pleurodema kriegi*

ARGENTINA: **Córdoba province**: La Posta, Pampa de Achala (31°36'46"S, 64°52'29"W, 2151 m a.s.l.). Froglets (GIHRC: 956–959); Juvenils (GIHRC: 960–963); Males (GIHRC: 559–561, 563–571, 574, 576, 577, 757–759, 761–764, 863–865, 868, 889, 890, 953, 955); Females (GIHRC: 562, 572, 573, 760, 765, 767, 862, 946–948) (49 specimens).

#### *Pleurodema cordobae*

ARGENTINA: **Córdoba province**: Estancia Los Tabaquillos, Sierra de Comechingones (32°23'58"S, 64°55'35"W, 2105 m a.s.l.). Froglets (GIHRC: 964–968); Juvenils (GIHRC: 969,970); Males (GIHRC: 581–586, 588–594, 645–650, 652, 654, 656, 657, 768, 769, 805, 806, 892, 906, 933); Females (GIHRC: 595, 681, 832, 846, 847, 882, 887, 899, 907, 917, 919, 921, 928, 929, 931–934, 936, 938) (57 specimens).

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