Karyotype of *Liolaemus uspallatensis* Macola and Castro, 1982: The smallest chromosome number of the genera and its comparison with other taxa

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Abstract

Liolaemus uspallatensis is a lizard distributed in the west of Argentina at 2000-2500 m of altitude. Its reproduction is oviparous, and besides this, little is known about its biology and natural history. In the present work the karyotype of *Liolaemus uspallatensis* is defined and compared with other taxa of the genus. Conventional cytogenetic techniques are employed to analyze the chromosomes using intestinal cells. Three individuals from Uspallata (Mendoza, Argentina) were analyzed and the diploid number was 2n= 28, with 6 pairs of macrochromosomes and 16 microchromosomes. The macrochromosomes pairs #1, 3, 4, 5 and 6 are metacentric, being the morphology of pair #2 submetacentric. The microchromosomes present dot appearance with the exception of the pairs #7, 8 and 9 clearly metacentric- submetacentric. This is the unique 2n= 28 diploid number reported for *Liolaemus* and the minor chromosomal number defined for the genus. Then, the chromosome morphology and length was compared with the ones obtained for other taxa of the genus. The biology of this Andean lizard is poorly known and its karyotype was not previously informed.

Resumen

Liolaemus uspallatensis es una de las especies de lagarto que se distribuye a más altitud en el oeste argentino (2000 a 2500 m.s.n.m.), ocupando ambientes de vegetación xerófila, típica de la formación del Monte. Se utilizaron para el análisis cromosómico tres individuos provenientes de las cercanías de la localidad de Uspallata (Mendoza, Argentina). Las preparaciones cromosómicas fueron obtenidas por raspado de epitelio intestinal y técnicas citogenéticas convencionales y el análisis de los cromosomas reveló un número diploide 2n= 28, con 6 pares de macrocromosomas y 16 microcromosomas. Los macrocromosomas presentaron los pares número (#)1, #3, #4, #5 y #6 con morfología metacéntrica y el par #2 submetacéntrica. Los pares (#7, #8 y #9) de microcromosomas presentaron morfología metacéntrica o submetacéntrica y el resto se describió con morfología puntiforme. Este es el único 2n= 28 informado para el género y el de menor número de cromosomas descripto hasta el momento por lo que se comparó la morfología y tamaño de los cromosomas con los obtenidos en otras especies de *Liolaemus*. Es aún muy escaso el conocimiento que se tiene sobre esta lagartija andina y su cariotipo no estaba descripto hasta el presente trabajo.

Introduction

The genus *Liolaemus* include small an median lizards whose distribution, endemic to South America, ranges from the cordilleras of Perú and Bolivia to Tierra del Fuego, from the Pacific Ocean coast through Argentina to the Atlantic Ocean including Uruguay and Southeastern of Brazil. The genus *Liolaemus* has more than 160 species described (Etheridge, 1995). Due the politomy of the genus the incorporation of no conventional morphological characters like cytogenetics ones can provide support to the knowledge of the phylogenetic relationships among the species. Only 53 species (33%) have the karyotype defined, 99% performed with direct methods and Giemsa conventional stain. These works provide the chromosome number and the morphology (Espinoza and Formas, 1976; Lamborot *et al*., 1979; Navarro *et al*., 1981; Sallaberry *et al*., 1982; Lamborot and Alvarez Sarret, 1989; Navarro Barón, 1991; Nuñez *et al.*, 1991; Hernando, 1992; Nuñez and Navarro, 1992; Navarro and Nuñez, 1992; Lamborot, 1993; Iturra *et al.,* 1994; Bunge and Quatrini, 1996; Hernando, 1996; Verrastro, 1996; Viña Bertolotto et al., 1996; Quatrini *et al.,* 1997; Aiassa *et al*. 1998[a], 1998[b], 1999, in press).

Differential stain was only reported for *L. chacoensis* with C bands (Hernando, 1996) and *L. occipitalis, L. lutzae* and *L. wiegmannii* with R bands (Viña Bertolotto *et al*., 1996).

In 1981, R. F. Laurent found in the collection of the Institute of Animal Biology, Mendoza (Argentina), some specimens of *Liolaemus*, without description, and he supposed to be *Liolaemus darwini*. Next year, Macola and Castro (1982) gave a brief observation of *Liolaemus uspallatensis* with a subsequent detailed description by Laurent (1984) and additional data provided by Cei (1986), and Scolaro and Cei (1991). However *Liolaemus uspallatensis* is still a poorly known Andean lizard (Scolaro and Cei, 1991). *Liolaemus uspallatensis* remains now from a few localities in the “Sierra de Uspallata”, Mendoza (Etheridge, 1993). Is one species of the “*L. darwinii complex*” (Etheridge, 1993) with high altitude distribution (2000-2500 m), on typical vegetation of the Monte geographycal form.

Medium size and tail/ total length is 65 mm. Males are polymorphic, with one pattern predominating: a deep chestnut color speckled with many scales of sky-blue, some creamy-yellow, some bricked-red scales on the posterior extremities and tail. Ventral surface of head is dark brownish-black, femoral region has scales bordered with yellow, dorsum crossed with obscure bands, and a black spot or "femoral patch" is evident in the humeral, region characteristic of the taxon (Etheridge, 1993).

L. uspallatensis was found to be confused for years with the sympatric species *Liolaemus ruibali* (Cei, 1986).

The objective of the present work is to report the karyotype of *L. uspallatensis* and to compare it with others taxa of the genus.

Material and method

Cytogenetic analysis was performed in three male individuals of *L. uspallatensis* from Uspallata (Mendoza, Argentina). Voucher specimens were deposited in the collection of the Laboratory of Zoology, Universidad Nacional de Río Cuarto: ZV-UNRC 3983, ZV-UNRC 3984 y ZV-UNRC 3985. Animals were handled according to the Institute normatives.

Chromosomes were obtained by scraping the intestine epithelial, and conventional cytogenetics smears were obtained using colchicine, hypotonic treatment, air drying, fixation and Giemsa stain (Aiassa et al., 1998 [b]). Selected metaphases from each specimen were photographed and several karyotypes were constructed.

Millimetric measurements were obtained in the three best metaphases from *L. uspallatensis* of the present work, *L. saxatilis* and *L. fitzgeraldi* of previous works (Aiassa et al., 1998[a], 1998[b]) and from *L. koslowskyi,* and *L. darwini* previously reported (Aiassa et al., 1999, in press). In the macrochromosomes the total and arm length of the chromosomes was measured and the relation between arm *r*: length of large arm (q)/ length of short arm (p) and the centromeric index *i*: 100p/(p

+q) were determined. It was considered metacentric (M) the chromosome with *r* between 1-1.5 and *i* 50-40, submetacentric (SM) for *r* between 1.51-7 and *i* 39.9-12.5 and acrocentric- telocentric (A-T) for *r* between 7.1-∞ and *i* 12.4-0, adapted from Levan *et al*., 1964. Results were expressed as media ± standard deviation.

Because of the small presentation, the morphology of the microchromosomes was determined through the microscopic observation. When is was possible to observe both chromosome arms of similar length with the centromere in a median position, metacentric- submetacentric (M-SM) morphology was assigned, when a single chromosome arm was observed the chromosome was considered acrocentric- telocentric (A-T). Chromosomes with dot appearance were considered puntiform (P).

The length of the chromosomes # 1 to 9 was individually measured and expressed as percentage of relative length (RL%) in respect of the total chromosome complement length. In the microchromosomes, the RL% of the total of puntiforms and RL% of each puntiform was calculated.

r and *i* were the X ± SD of three metaphase measurements and Friedman test was performed for the differences among percentages of relative lengths of the five species of *Liolaemus* compared (PRISM, 1997). The chromosome measurements obtained in the five species of Liolaemus were analyzed in relation to chromosome evolution.

Results

The 33 metaphases of *Liolaemus uspallatensis* analyzed had a diploid number 2n= 28, with six pairs of macrochromosomes and sixteen microchromosomes (Fig. 1).

In table 1 are showed: *r, i*, relative length % and chromosome morphology of *Liolaemus uspallatensis*. In the macrochromosomes, the pairs number (#) 1, #3, #4, #5 and #6 (*r* between 1,06 y 1,45) are metacentric- submetacentric and pair #2 (*r:* 1,77) submetacentric. In this pair, a terminal secondary constriction was observed in the large arm. The macrochromosomes represent a 70,89% of the

total genome. In the microchromosomes the first three pairs (#7, #8 and #9) have metacentric- submetacentric appearance at microscope. The total of microchromosomes occupies the 29,11% of the genome and the pair number #7 takes the 40% of the first three pairs of microchromosomes.

In table 2 the relative length percentages and the chromosome morphology of *L. uspallatensis* (2n= 28), *L. koslowskyi* (2n= 36)*, L. darwini* (2n= 34) *L. saxatilis* (2n= 32) and *L. fitzgeraldi* (2n= 30) are compared. In the macrochromosomes, the morphology of pairs #1, #3, #4 and #5 are M and the pair #2 is SM in all the species. While pair #6 is SM in *L. koslowskyi* and *L. saxatilis*; the morphology is M in *L. darwini*, *L. fitzgeraldi* and *L. uspallatensis*. With respect to the variations of the first three pairs of microchromosomes *L. uspallatensis* presents the pair #7 with M-SM morphology and in the rest of the species is A-T. The pairs #8 and #9 are M-SM in all the species considered, with the exception of pair #9 which morphology is A-T in *L. koslowskyi*. The whole macrochromosomes occupy the 77.11 $\pm$ 1.85% of the genomes. The first three pairs of microchromosomes #7, #8 and #9 in *L. uspallatensis* takes the 13,22% of the total, significantly larger ($P< 0.01$) than *L. koslowskyi , L. darwini*, *L. saxatilis* and *L. fitzgeraldi* (Table 2*)*. Each puntiform of *L. uspallatensis* is also significantly larger than in the rest of the species. The total of puntiforms occupies from 11,2% en *L. fitzgeraldi* to 15,89% in *L. uspallatensis*.

Discussion

The chromosome number 2n= 28 is not reported, before now, for the *Liolaemus* genus, which diploid numbers ranges from 2n= 30 to 2n= 44 (Paull et al., 1976; Lamborot, 1993). Since *L. uspallatensis* it is possible to affirm that the variation of the chromosome number in the genus is between 2n= 28 and 2n= 44.

The morphology of the macrochromosomes # 1 to 5 is similar to the pattern observed for the species of *Liolaemus* with "conserved" karyotype, without changes through chromosome evolution

In our experience with the cytogenetics of the genus *Liolaemus,* the most distinctive difference between the karyotype of *L. uspallatensis* and other taxa is the length of the first three pairs of microchromosomes and the puntiforms. At the light microscope these have an appearance notably larger than the previous studied *Liolaemus.* To make an objective observation of this appreciation, millimetric measurements of the chromosomes of *L. uspallatensis* were compared with other four species (Table 2).

From the comparisons of the millimetric measurements of *L. uspallatensis* 2n= 28 (present work), with *L. koslowskyi,* 2n= 36 (Aiassa *et al*., in press), *L. darwinii,* 2n= 34 (*Aiassa et al*., 1999) previously reported; and *L. saxatilis*, 2n=32, *L. fitzgeraldi,* 2n= 30 obtained from previous works (Aiassa *et al*., 1998 [a], 1998 [b]), we can assume that the differences are produced by the morphology of the macrochromosome #6 and by the number, morphology and length of the microchromosomes.

Chromosomes # 1, 3, 4and 5 are M and #2 is SM in all the species. Pair #6 is M in some species and SM in others, then it is supposed to be the most susceptible of the macrochromosomes to chromosome rearrangements involved in the chromosome evolution of the genus. This was also observed for other authors in populations of *L. monticola* (Lamborot, 1993). In opposition to this, the pairs #1, #2, #3, #4 and #5 would be the most constants or "conserved".

With respect to the microchromosomes, the first three pairs (#7, 8 and 9) in *L. uspallatensis* are 61% larger than in others *Liolaemus*, the total of RL% in pairs #7,

#8 and #9 is 13.22%, being 8.13 % ± 0.28 in the other taxa. Besides this, each one of the puntiforms of *L. uspallatensis* is 55 % larger than in the other species.

The differences in the diploid numbers in the considered species seems to be produced by rearrangements in the microchromosomes and not in the macrochromosomes. The one which present the minor chromosome number (*L. uspallatensis* 2n= 28) has each microchromosome with RL% significantly larger respect of the other species, without significant variations in the macrochromosomes sizes. The relative length % of the total of macrochromosomes, is similar in all the compared species, a mean of 77.11% ± 1.85 of the total genome.

In relation to the morphology of the first three pairs of microchromosomes, the species with the larger chromosome number (*L. koslowskyi* 2n= 36) has two pairs A-T. Besides this, the one with minor diploid number (*L uspallatensis* 2n= 28), has the three pairs with M-SM morphology, and the other species one pair A-T and two pairs M-SM. Both previous observations support the hypothesis that inter-specific variations are favored by fusion- fission rearrangements and pericentric inversions in the chromosomes (Lamborot and Alvarez Sarret, 1989, Lamborot, 1991, 1993).

The cytogenetic studies with differential staining are scarce in the genus. Of the 53 species citogenetically studied it was reported C band in *L. chacoensis* (Hernando, 1996), *L. occipitalis, L. lutzae* and *L. wiegmannii* and only in *L. lutzae* it is completed with R band, in fibroblast cultures (Viña Bertolotto et al., 1996). C and G banding was performed in the present material, without results. A possible explanation would be the homogeneity in the composition of base pairs of the DNA in the great part of the reptiles, not compartmentalized in bands rich in AT or CG like in other vertebrates (Schmidt et al., 1990).

It is evident the difficulty to obtain bands in this material so the use of millimetric measurements is an alternative tool in the description and comparison of different karyotypes. This has also been employed in Amphibia (Bogart, 1972), and provides new morphological characters for philogenetic studies.

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Table 1**:** Relation between arms, centromeric index, chromosome relative length and chromosome morphology of *Liolaemus uspallatensis* (2n= 28).

Chromosome pair	*r**	*i**	Percentage of relative length (%)	Chromosome morphology
1	1.11 ± 0.13	47.47 ± 2.93	15.98	M
2	1.77 ± 0.16	36.18 ± 2.08	15.13	SM
3	1.45 ± 0.22	46.87 ± 4.53	12.17	M
4	1.13 ± 0.16	46.16 ± 3.34	11.53	M
5	1.06 ± 0.11	48.57 ± 2.48	9.10	M
6	1.11 ± 0.19	47.62 ± 4.12	6.98	M
7	Nd	Nd	5.29	M-SM
8	Nd	Nd	4.23	M-SM
9	Nd	Nd	3.70	M-SM
10-14	Nd	Nd	15.89	P

r: length of large arm/ length of short arm, *i*: centromeric index, Nd: not determined, M: metacentric, SM: submetacentric, P: puntiform, * X±SD of three metaphase measurements.

Table 2: Comparison of the chromosome relative lengths and chromosome morphologies among *Liolaemus* species

Species / locality		*L. koslowskyi* / Anillaco-La Rioja	*L.darwinii* / Cutral Có-Neuquén	*L. saxatilis* / Achiras-Córdoba	*L. fitzgeraldi* / Calingasta-San Juan	*L.uspallatensis* / Uspallata-Mendoza
2n		36	34	32	30	28
Reference		Aiassa et al., in press	Aiassa et al., 1999	Aiassa et al., present work	Aiassa et al., present work	Aiassa et al., present work
PAIR		Percentage of relative lengths (RL%)/ morphology				
MACs	1	16.90/ M	18.37/ M	19.45/ M	19.10/ M	15.98/ M
	2	17.10/ SM	17.15/ SM	16.60/ SM	17.01/ SM	15.13/ SM
	3	13.05/ M	13.09/ M	14.73/ M	13.98/ M	12.17/ M
	4	12.39/ M	11.46/ M	12.77/ M	12.81/ M	11.53/ M
	5	9.41/ M	10.16/ M	10.66/ M	11.93/ M	9.10/ M
	6	6.76/ SM	7.23/ M	6.26/ SM	6.29/ M	6.98/ M
	T	75.61	77.46	80.47	81.12	70.89 ns
MICs	7	3.71/A-T	3.58/ A-T	3.09/A-T	2.79/ A-T	5.29/M-SM
	8	2.78/M-SM	2.60/M-SM	2.60/M-SM	2.79/M-SM	4.23/M-SM
	9	2.29/A-T	2.03/M-SM	1.95/M-SM	2.10/M-SM	3.70/M-SM
	T	8.78	8.41	7.64	7.68	13.22**
	P_t	15.51	14.33	11.89	11.2	15.89
	Each P	0.86	0.89	0.85	0.93	1.59**

M: metacentric, SM: submetacentric, t: total, P: puntiforms, A-T: acrocentric-telocentric, MACs: macrochromosomes, MICs: microchromosomes, ns: not significant, ** P< 0.001 for these values, Friedman test.

Figure 1: a- Metaphase of *Liolaemus uspallatensis* (male)

b- Karyotype of *Liolaemus uspallatensis* (male)