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C-BANDING PATTERNS AND MEIOTIC BEHAVIOR IN *HYPSIBOAS PULCHELLUS* AND *H. CORDOBAE* (ANURA, HYLIDAE)

Baraquet M.[1,2], Salas N.E.[1], Martino A.L.[1]

[1] Ecología, Departamento de Ciencias Naturales, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto. Ruta Nacional N° 36 - km 601, (X5804BYA) Río Cuarto, Argentina.
[2] CONICET

mbaraquet@exa.unrc.edu.ar

ABSTRACT

In this work, we observed and evaluated C-banding patterns and meiotic behaviour of *Hypsiboas pulchellus* and *H. cordobae*. Twenty metaphasic cells per individual were analyzed, which were obtained from intestinal and testis cells using conventional and C-banding techniques. The two species presented *2n*= 24 chromosomes. Pairs 1 and 8 to 12 were metacentric whereas pairs 2 to 7 were submetacentric. The centromeric region of all chromosomes was positively C-banded but the C-banding pattern varied between species. At meiosis, cells with 12 bivalents were observed. Bivalents usually presented two terminal chiasmata, although the largest pair presented only one terminal chiasma. The cytogenetic data reconfirms the diploid somatic chromosome number of *H. pulchellus* and *H. cordobae*, species that exhibited identical chromosome morphology. The remarkable similarities between the two species are an indication of the close relationship among members of the *H. pulchellus* group; however C-banding patterns of *Hypsiboas* species are distinct and may be also referred to as species-specific. Meiotic behavior is quite similar to the behaviour of most frogs, which possess only one or two -usually terminal- chiasmata, resulting in the typical linear or ring appearance of bivalents.

Key words: Karyotypes, C-banding, Meiosis, *Hypsiboas pulchellus, Hypsiboas cordobae.*

RESUMEN

Este trabajo describe el patrón de Bandas-C y comportamiento meiótico de *Hypsiboas pulchellus* e *H. cordobae*. Se analizaron 20 figuras metafásicas por individuo, obtenidos de intestinos y testículos utilizando técnicas convencionales y de bandeo-C. Para ambas especies se determinó un número cromosómico de *2n*= 24. Los pares 1 y 8 a 12 mostraron morfología metacéntrica, y los pares 2 a 7 mostraron morfología submetacéntrica. Para las dos especies se encontró el mismo patrón de heterocromatina constitutiva en la región centromérica de todos los pares, pero el patrón de bandeo varió entre ellas. En meiosis se observaron células con 12 bivalentes. Los bivalentes mostraron normalmente dos quiasmas, pero en el bivalente mayor también se observó un único quiasma terminal. Los datos citogenéticos reconfirman el número diploide de *H. pulchellus* e *H. cordobae*, y muestran una morfología de los cromosomas idéntica para ambas especies, siendo esta similitud un indicio de la estrecha relación entre las especies del grupo *H. pulchellus*; sin embargo los patrones de Bandas-C de las dos especies de *Hypsiboas* son distintos, pudiendo ser esto reflejo de un carácter especie-específico. El comportamiento meiótico es similar al encontrado en otras especies de anuros por diversos autores.

Palabras clave: Cariotipos, Bandas-C, Meiosis, *Hypsiboas pulchellus*, *Hypsiboas cordobae*.

INTRODUCTION

The genus *Hypsiboas* Wagler (1830), which belongs to the Hylidae family, Hylinae subfamily and Cophomantini tribe, contains 84 species, most of them included in seven species groups. One of these is the *Hypsiboas pulchellus* group, which currently contains 36 species (Faivovich *et al.*, 2004; Faivovich *et al.*, 2005; Frost, 2012; Köhler *et al.*, 2010; Lehr *et al.*, 2010; Lehr *et al.*, 2011). This group includes *Hypsiboas pulchellus* (Duméril and Bibron, 1841) and *Hypsiboas cordobae* (Barrio, 1965), which are the object of the present study.

H. pulchellus is a widely distributed amphibian, occurring from Santa Catalina to Rio Grande do Sul in Brazil and in Uruguay, and also in the Argentinian provinces of Misiones, Corrientes, Entre Ríos, Santa Fe, La Pampa, Chaco, Córdoba, Buenos Aires and northern Río Negro. Its sister species, *H. cordobae*, is also found in Argentina, in the hills of Córdoba and San Luis provinces (Barrio, 1962; 1965; Cei, 1980; Gallardo, 1974; 1987; Basso and Basso, 1987; Basso, 1990; Martori and Ávila, 1992; Bridarolli and di Tada, 1994; di Tada *et al.*, 1996; Ávila *et al.*, 1999; di Tada, 1999; Langone and Lavilla, 2002; Faivovich *et al.*, 2004; Kwet *et al.*, 2004), but the exact limits of its range remain unknown, especially in the contact area with *H. pulchellus* (Barrio, 1965; Cei, 1980; Gallardo, 1987; Bridarolli and di Tada, 1994; Stuart, 2006).

These two species are similar in morphology and ecological features, and have been cytogenetically analyzed by various authors (Morescalchi, 1973; King, 1990; Barale *et al.*, 1991). Although the number of cytogenetic studies seems reasonable, the majority of them are reports of chromosome numbers and morphological phenotypes. Because chromosome studies may be of help to clarify phylogenetic and taxonomic relationships (Reyes Valdéz *et al.*, 2000), in the present study we redescribe the karyotypes *H. pulchellus* and *H. cordobae* with the aim of providing new data for the analysis of meiotic behavior and C-banding patterns.

MATERIALS AND METHODS

Our sample of *H. pulchellus* comprises 18 males specimens collected from different populations in Córdoba province: Río Cuarto (33° 06' 40.78" S, 64° 18' 16.88" W), Las Acequias (33° 15' 26.16" S, 63° 55' 15.10" W), Alejandro Roca (33° 21' 06" S, 63° 42 '10" W). Thirty-two males specimens of *H. cordobae* were collected from different populations in Córdoba and San Luis provinces: Achiras (33° 09' 28.64" S, 64° 58' 55.13" W), Las Guindas (32° 35' 35.22" S, 64° 42' 38.92" W), Pampa de Achala (31° 49' 41.8" S, 64° 51' 44.9" W), Los Linderos (32° 00' 54.05" S, 64° 56' 42.97" W), Los Tabaquillos (32° 23' 59.75" S, 64° 55' 33.69" W), La Carolina (32° 48' 43.94" S, 66° 05' 48.15" W).

Chromosome preparations were obtained as described by Baraquet *et al.* (2011), and by Schmid (1978), Schmid *et al.* (1979), Salas (2006) and Salas and Martino (2007). C-banding was done according to Sumner (1972).

Chromosomes were visualized using a Zeiss Axiophot-Axiolab and photographed using Axiocam HRc Zeiss. We analyzed 20 metaphasic cells per individual. On the metaphases the total length of each chromosome and the length of the four arms were measured using image analysis by Adobe® Photoshop® 9.0.

Data were processed using Microsoft Excel® 2000. Length of *p* and *q* arms, centromeric index, arm ratio, relative chromosome length and relative arm length were calculated using the following formulas:

Average length of arms *p* y *q*: $q = (q^1 + q^2)/2$

$p = (p^1 + p^2)/2$

Centromeric index (*i*): i = (length of the short arm of a chromosome (p) / total length of the chromosome ($p + q$)) × 100

Arm ratio (*r*): $r = q/p$

Relative chromosome length (*rl*): rl = (total length of chromosome / $\sum$ of length of chromosomes of the haploid set) × 100

Relative arm length (*rlq* o *rlp*): rlq or rlp = (length of the arms (q o p) / $\sum$ of length of chromosome of the haploid set) × 100

The data were processed to get the average for each species. The ideograms were carried out with these data, which was necessary because in the karyotypes the differences between chromosomes are not observable to the naked eye.

The chromosomes were classified according to Aiassa *et al.* (2001).

RESULTS

All populations of *H. cordobae* and *H. pulchellus* had *2n*= 24 chromosomes with a fundamental number NF= 48 (Figure 1). The twelve chromosome pairs can be classified into three groups. The chromosomes of pair 1 are large with a relative chromosome length of 16.20 % for *H. cordobae* and 12.26 % for *H. pulchellus*. Pairs 2 to 6 comprise a group of medium chromosomes with a relative chromosome length between 12.45 % and 7.58 % for *H. cordobae* and 10.63 % and 8.79 % for *H. pulchellus*. The remaining six chromosome pairs comprise a group of small chromosomes with a relative chromosome length between 6.57 % and 4.04 % for *H. cordobae* and 8.03 % and 3.90 % for *H. pulchellus* (karyotype formula 1: 5: 6) (Table 1, Figures 1, 2).

The chromosome morphology was always metacentric or submetacentric in the two species studied. Pairs 1 and 8-12 were metacentrics whereas the pairs 2-7 were submetacentrics.

C-banding showed the same pattern of constitutive heterochromatin at the centromeric regions of all pairs in the *H. pulchellus* and *H. cordobae*.

In *H. pulchellus* pericentromeric heterochromatin in all pairs, except pair 8, was observed. C-banding in the interstitial regions of the long arms of the pair 2 was observed. C-banded regions also appeared on the telomeric regions of the short arms of the pairs 1, 3, 5, 7 and 8, and in the long arms of the pairs 1-10 (Figure 3A y Table 2).

In *H. cordobae* revealed positively stained pericentromeric heterochromatin in the chromosomes of pairs 4, 5 and 8-12. C-banding in the interstitial regions of the short arms of the pair 1 was observed (Figure 3B y Table 2).

The meiotic analysis of males showed that the two species studied had 12 bivalents at diakinesis and 12 chromosomes at metaphase II cells (Figure 4A-D). The bivalents generally had two terminal chiasmata (ring-shaped bivalents).

In *H. cordobae* in most of the cells analyzed (78.50 %) the larger bivalent had one terminal chiasma (Figure 4C). This meiotic configuration was observed in the six populations studied; however, in the population of Pampa de Achala many of the cells exhibit the twelve bivalents ring-shaped (two terminal chiasmata). However, in *H. pulchellus*, most of the cells analyzed (69.23 %) exhibit all ring-shaped bivalents (Figure 4A) and only in Río Cuarto population the larger bivalent had one terminal chiasma in half of the cells (Table 3).

Table 1. Karyotype parameters for constructing *H. pulchellus* and *H. Cordobae ideograms*

P	*H. pulchellus*				*H. cordobae*			
	rl	*r*	*i*	T	*rl*	*r*	*i*	T
1	12.26	1.09	47.88	M	16.20	1.18	45.94	M
2	10.63	1.52	39.72	SM	12.45	1.71	37.74	SM
3	10.30	1.55	39.33	SM	11.33	2.14	33.26	SM
4	10.30	2.89	25.89	SM	10.55	2.73	27.81	SM
5	9.65	2.03	33.06	SM	9.54	2.59	31.81	SM
6	8.79	2.78	24.45	SM	7.58	2.95	28.53	SM
7	8.03	1.94	34.46	SM	6.57	1.94	35.08	SM
8	7.70	1.43	41.25	M	5.99	1.51	40.15	M
9	6.51	1.26	44.41	M	5.52	1.41	41.61	M
10	6.18	1.27	44.15	M	5.31	1.40	43.54	M
11	5.75	1.06	48.45	M	4.81	1.34	42.86	M
12	3.90	1.10	47.62	M	4.04	1.24	44.97	M

P: number of chromosome pairs; *rl*: chromosome relative length; *r*: arm ratio; *i*: centromeric index; T: Type, M: metacentric, SM: submetacentric.

Table 2. Distribution of heterochromatin in *H. pulchellus* and *H. cordobae.*

	H. pulchellus					*H. cordobae*		
P	**C**	**PC**	**BIq**	**Tp**	**Tq**	**C**	**PC**	**BIp**
1	+	+		+	+	+		+
2	+	+	+		+	+		
3	+	+		+	+	+		
4	+	+			+	+	+	
5	+	+		+	+	+	+	
6	+	+			+	+		
7	+	+		+	+	+		
8	+			+	+	+	+	
9	+	+			+	+	+	
10	+	+			+	+	+	
11	+	+				+	+	
12	+	+				+	+	

P: number of chromosome pairs; C: bands in centromeric regions; PC: bands in pericentromeric regions; BIp: bands in interstitial regions of short arms; BIq: bands in interstitial regions of long arms, Tp: bands in telomeric regions of short arms; Tq: bands in telomeric regions of long arms.

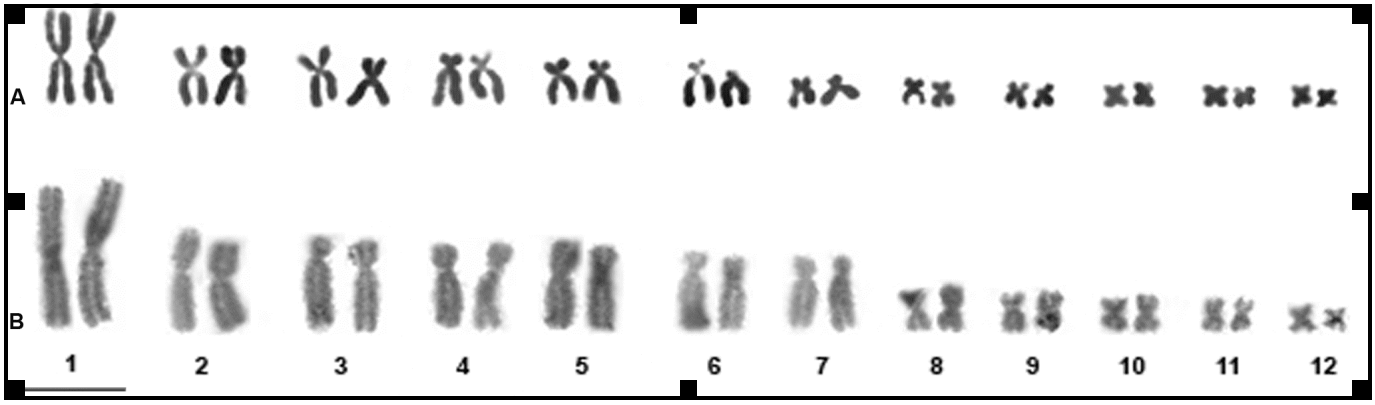

Figure 1. Karyotypes of *H. pulchellus* (A) and *H. cordobae* (B). (Bar=10 µm).

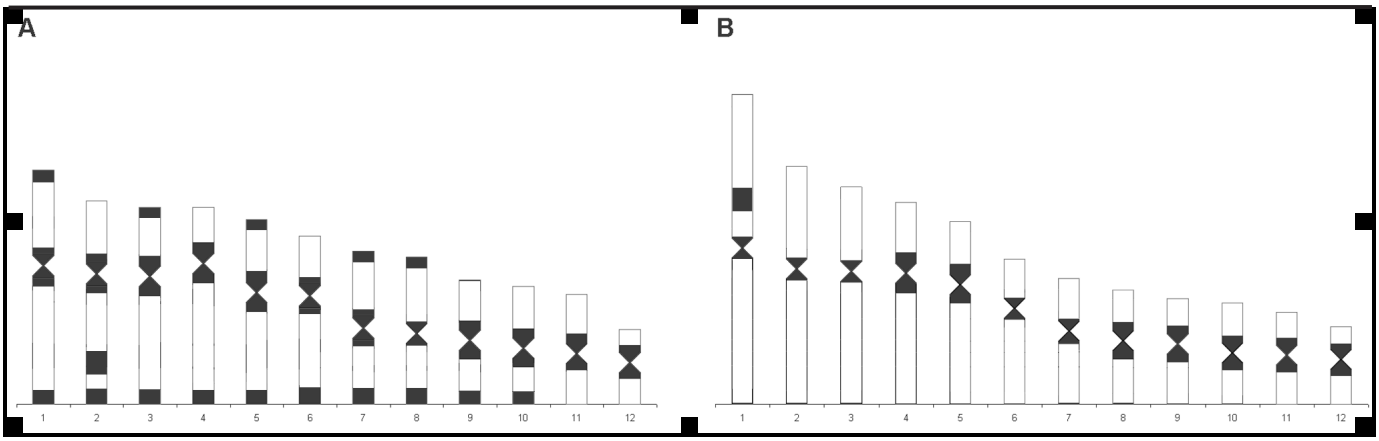

Figure 2. Karyotypes of *H. pulchellus* (A) and *H. cordobae* (B). (Bar=10 µm).
Ideograms based on the parameters presented in Table 1: *H. pulchellus (A)* and *H. cordobae* (B). Black areas indicate C-bands.

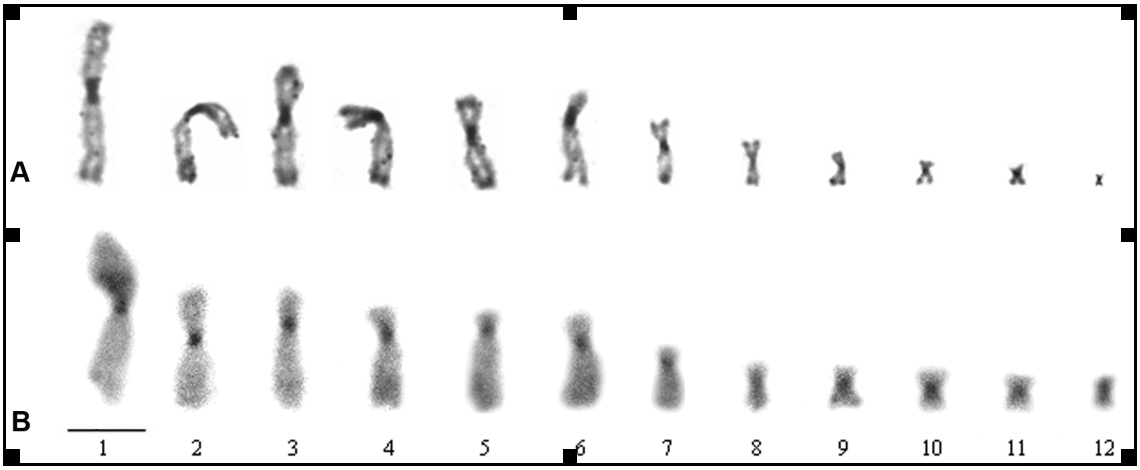

Figure 3. C-banded karyotypes of *H. pulchellus* (A) and *H. cordobae* (B). (Bar=10 µm)

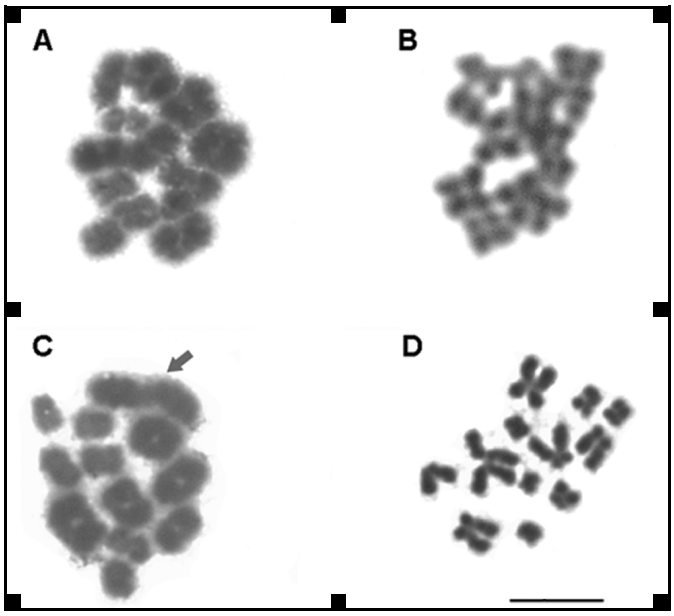

Figure 4. Meiotic cells of *Hypsiboas pulchellus* (A, B) and *H. cordobae* (B, C). (A) 12 bivalents with two terminal chiasmata. (B) Metaphase II with n= 12. (C) 11 ring-shaped bivalents and one bivalent with a terminal chiasma (arrow). (D) Metaphase II with n= 12 (D). (Bar=10 µm).

DISCUSSION

It is well known that most of Hylinae subfamily species have diploid chromosome numbers between 22 and 34 but most of the species within the genus *Hypsiboas* have a diploid complement 2*n*= 24 (Morescalchi, 1973; King, 1990).

Despite the differences in morphology and advertisement call between *H. pulchellus* and *H. cordobae* (Barrio, 1962; 1965; Cei, 1980; Basso and Basso, 1987; Gallardo, 1987; Faivovich *et al.*, 2004; Baraquet *et al.*, 2007), these species show the same diploid number (2*n*= 24) and a conserved chromosomal morphology.

Moreover, a comparison of known karyotypes of species of three of the five genus included in Cophomantini, *Aplastodiscus*, *Bokermannohyla*, and *Hypsiboas* corroborates the remarkable similarity among them (Catroli *et al.*, 2011).

The diploid number of *H. pulchellus* and *H. cordobae* is largely in accordance with those previously described in the literature for these species (Bogart, 1973; Morescalchi, 1973; Barale *et al.*, 1991; Ananias, 1996) and was identical to other species of *Hypsiboas pulchellus* group (*H. caingua*, *H. prasinus*, *H. joaquini*, *H. semiguttatus*, *H. marginatus*, *H. guentheri*, *H. bischoffi*) (Baldissera *et al.*, 1993; Ananias *et al.*, 2004; Raber *et al.*, 2004), and *Hypsiboas faber* group (*H. albomarginatus* and *H. faber*) (Carvalho *et al.*, 2009). In *Hypsiboas* the chromosome number and the morphology of the pairs is similar among species, with few or almost no differences among most of them. The only known species with a different chromosome number is *H. albopunctatus* with 2*n*= 22 (Gruber *et al.*, 2007; Catroli *et al.*, 2011).

About the chromosome morphology, metacentric or submetacentric for both species studied, our results are in agreement with King (1990). This author reported that *Hypsiboas* species with diploid complement 2*n*= 24 have metacentric or submetacentric karyotypes.

These results obtained here show that the two species have common features, however small differences in the chromosome size were observed.

The chromosome morphology of both species was similar to those reported by Barale *et al.* (1991) for *H. cordobae*. However, the metacentric morphology of pair 2 and subterminal morphology of pairs 4 and 6 reported by those authors is not observed for us in *H. cordobae.* For *H. pulchellus*, Ananias (1996) indicated submetacentric morphology in the pairs 9 and 10, and acrocentric morphology of the pair 6. However, our results did not show this structure.

According to Schmid *et al.* (1990), in the chromosomes of the Anura the centromeric/pericentromeric regions and telomeres are the preferential locations of heterochromatin. *H. cordobae* and *H. pulchellus* show the anuran pattern of constitutive heterochromatin: at centromeric regions in all chromosome pairs and at pericentromeric regions in some of them. We observed that the most important distinction between *H. pulchellus* and *H. cordobae* karyotypes was in the C-banding pattern.

Also, the comparison of the C-banding patterns of the two species studied with those previously described for the *Hypsiboas pulchellus* group and related species reveal that there are some common C-bands in most of them. *H. prasinus* (Baldissera, *et al.* 1993; Ananias, 1996), *H. joaquini* (Ananias, 1996), *H. guentheri* and *H. bischoffi* (Raber *et al.*, 2004), *H. marginatus* and *H. semiguttatus* (Ananias *et al.*, 2004) have a telomeric band in the long arm of chromosome 1 as in *H. pulchellus*. The C-banded chromosomes of *H. pulchellus* revealed heterochromatin at the pericentromeric regions of all pairs (except the pair 8) as in *H. guentheri* and *H. bischoffi* (Raber *et al.*, 2004). However, the telomeric heterochromatic band on the long arm of pair 10 observed in *H. pulchellus*, *H. guentheri* and *H. bischoffi* (Raber *et al.*, 2004), *H. marginata* and *H. semiguttata* (Ananias *et al.*, 2004), is not observed in *H. cordobae*.

Chromosome banding is a very important tool in comparative cytogenetics (Baldissera *et al.*, 1993). Interspecific comparisons of C-band patterns are a basic importance for cytotaxonomic studies (Schmid, 1978; Schmid *et al.*, 1990; Baldissera *et al.*, 1993). It has been shown that although many species of several amphibian genuses have a shared similarity in chromosome number and morphology, as we observed among the studied species, could be extensive differences in the position and amount of heterocromatin (Schmid, 1978; Schmid, *et al.*, 1990).

Regarding to meiosis, at diakinesis in both species 12 ring-shaped bivalents are observed, but frequently the larger bivalent present only one terminal chiasmata. These meiotic configurations have also been described by Baldissera *et al.* (1993) for four Brazilian *Hyla* species; by Martirosyan and Stepanyan (2007) for *Hyla savignyi*; and in diplotene cells of *Hypsiboas albopunctatus* (Gruber *et al.*, 2007). Also, Lourenço *et al.* (2003) observed in *Paratelmatobius cardosoi* (Leptodactylidae) that a high percentage of diakinesis showed the larger bivalent with only one terminal chiasmata and open configuration, as observed in this study. These authors explain that this open configuration probably is the result of a terminal association of the long arms of pair 1. The two species studied are quite similar from most frogs, which possess only one or two, usually terminal, chiasmata, resulting in the typical linear or ring appearance of bivalents (Morescalchi, 1973).

The cytogenetic data obtained in this paper reconfirms the diploid number of *H. pulchellus* and *H. cordobae* and show identical chromosome morphology; however we find that these species differ slightly in the heterochromatin pattern.

It has been noticed that most members of Cophomantini, and specifically members of the *H. pulchellus* group (Ananias *et al.*, 2004; Raber *et al.*, 2004), share a similar chromosome morphology (Gruber *et al.*, 2007; Catroli *et al.*, 2011), and therefore, the remarkable similarities are no more than an indication of near relationship between *H. cordobae* and *H. pulchellus*. Even these similarities could be indication of a relationship between any member of the *H. pulchellus* group or with almost any other species of *Hypsiboas*, with the exception of some members of the *H. albopunctatus* group (Bogart, 1973; Gruber *et al.*, 2007).

This work represents a significant contribution to redescription of the karyotype of *H. pulchellus* and *H. cordobae*, and a contribution with new data of meiotic behavior and C-banding patterns, not made so far.

REFERENCES

Aiassa D., Gorla N., Ávila L., Martori R. (2001) Cariotipo de *Liolaemus koslowskyi* Etheridge, 1993. Nuevo número cromosómico para el género (2n = 36). Rev. Esp. Herp. 15: 37-43.

Ananias F. (1996) Caracterizaçao cromossômica de espécies e subespécies de *Hyla* do grupo *pulchella* (Amphibia, Anura, Hylidae). Master thesis. State Univ. of Campinas (UNICAMP), SP, Brazil.

Ananias F., García P.C.A., Recco-Pimentel S.M. (2004) Conserved karyotypes in the *Hyla pulchella* species group (Anura, Hylidae). Hereditas 140: 42-48.

Ávila L.J., Morando M., Priotto J.M. (1999) Amphibia and Reptilia of the high grasslands of the Sierra de Comechingones, Córdoba, Argentina. B. Md. Herpetol. Soc. 35: 97-113.

Baldissera Junior F.A., Lopez de Oliveira P.S., Kasahara S. (1993) Cytogenetics of tour Brazilian *Hyla* species (Amphibia-Anura) and description of a case with a supernumerary chromosome. Rev. Brasil. Genet. 16: 335-345.

Barale G.D, di Tada I.E., Lisanti J.A. (1991) Descripción del cariotipo y ubicación del organizador nucleolar en *Hyla pulchella cordobae* (Anura, Hylidae) de la Pampa de Achala. Rev. UNRC 11: 31-34.

Baraquet M., Salas N.E., di Tada I.E. (2007) Variación geográfica en el canto de advertencia de *Hypsiboas pulchellus* (Anura, Hylidae) en Argentina. Rev. Esp. Herp. 21:107-118.

Baraquet M., Valetti J.A., Salas N.E., Martino A.L. (2011) Redescription of the karyotype of five species of the family Bufonidae (Amphibia: Anura) from central area of Argentina. Biologia 66 (3): 543-547.

Barrio A. (1962) Los Hylidae de Punta Lara, Provincia de Buenos Aires. Observaciones sistemáticas, ecológicas y análisis espectrográfico del canto. Phycis 23: 129-142.

Barrio A. (1965) Las subespecies de *Hyla pulchella* Duméril & Bibron (Anura, Hylidae). Phycis 25: 115-128.

Basso N.G. (1990) Estrategias adaptativas en una comunidad subtropical de anuro. Cuad. Herpetol. Serie monografías Nº 1.

Basso N.G., Basso G. (1987) Análisis acústico del canto de *Hyla pulchella pulchella* (Duméril & Bibron, 1841) (Anura: Hylidae). An. Mus. Hist. Nat. Valparaiso 18: 109-114.

Bridarolli M.E., di Tada I.E. (1994) Biogeografía de los Anfibios Anuros de la Región Central de la República Argentina. Cuad. Herpetol. 8: 63-82.

Bogart J.P. (1973) Evolution of anuran karyotypes. In: Vial J.L. (Ed.) Evolutionary biology of anurans. Univ. Missouri Press, pp. 337-349.

Carvalho K.A, Garcia P.C.A., Recco-Pimentel S.M. (2009) Cytogenetic comparison of tree frogs of the genus *Aplastodiscus* and the *Hypsiboas faber* group (Anura, Hylidae). Genet. Mol. Res. 8 (4): 1498-1508.

Catroli G.F., Faivovich J., Haddad C.F.B, Kasahara S. (2011) Conserved Karyotypes in Cophomantini: Cytogenetic Analysis of 12 Species from 3 Species Groups of *Bokermannohyla* (Amphibia: Anura: Hylidae). J. Herpetol. 45: 120-128.

Cei J.M. (1980) Amphibians of Argentina. Monit. Zool. Ital. (N.S.) 2: 1-609.

Di Tada I.E., Zavattieri M.V., Salas N.E., Martino A.L. (1996) Anfibios Anuros de la provincia de Córdoba. In: di Tada I.E., Bucher E.H. (Eds.) Biodiversidad de la provincia de Córdoba. Volumen I. Fauna. U.N.R.C., pp. 191-215.

Di Tada I.E. (1999) Patrones de distribución de los anfibios anuros de la provincia de Córdoba. Tesis Doctoral, Facultad de Ciencias Exactas, Físicas y Naturales, U.N.C., Córdoba, Argentina.

Faivovich J., García P.C.A., Ananias F., Lanari L., Basso N.G., Wheeler W.C. (2004) A molecular perspective on the phylogeny of the *Hyla pulchella* species group (Anura, Hylidae). Mol. Phylogenet. Evol. 32: 938-950.

Faivovich J., Haddad C.F.B, García P.C.A., Frost D.R., Campbell J.A., Weeler W.C. (2005) Systematic review of the frog Family Hylidae, with special reference to Hylinae: Phylogenetic analysis and taxonomic revision. B. Am. Mus. Nat. Hist. 294: 1-240.

Frost D.R. (2012) Amphibian Species of the World: an online reference. Version 5.6. Am. Mus. Nat. Hist., New York, USA. http://research.amnh.org/vz/herpetology/amphibia/ (accessed October 2012).

Gallardo J.M. (1974) Anfibios de los alrededores de Buenos Aires. Editorial Universitaria de Buenos Aires, Buenos Aires.

Gallardo J.M. (1987) Anfibios Argentinos. Guía para su identificación. Biblioteca Mosaico, Lib. Agropecuaria, Buenos Aires 98: 81-82.

Gruber S.L., Haddad C.F.B., Kasahara S. (2007) Chromosome banding in three species of *Hypsiboas* (Hylidae, Hylinae), with special reference to a new case of B-chromosome in anuran frogs and to the reduction of the diploid number of 2n = 24 to 2n = 22 in the genus. Genetica 130: 281-291.

King M. (1990) Animal Cytogenetics. Vol. 4: Chordata 2. Amphibia. Gerbrüder Borntraeger Berlin Stuttgart.

Köhler J., Koscinski D., Padial J.M., Chaparro J.C., Handford P., Lougheed S.C., De La Riva I. (2010) Systematics of Andean gladiator frogs of the *Hypsiboas pulchellus* species group (Anura, Hylidae). Zool. Scr. 39: 572-590.

Kwet A., Aquino L., Lavilla E., di Tada I. (2004) *Hypsiboas pulchellus*. IUCN Red List de Threatened Species. Version 2009.1. http://www.iucnredlist.org/ (accessed June 2009).

Langone J.A., Lavilla E.O. (2002) Comentarios nomenclatoriales sobre algunos taxa del grupo de *Hyla pulchella* (Anura: Hylidae). Cuad. Herpetol. 16: 73-78.

Lehr E., Faivovich J., Jungfer K.H. (2010) A new Andean species of the *Hypsiboas pulchellus* group: adults, calls and phylogenetic relationships. Herpetologica 66: 296-307.

Lehr E., Faivovich J., Jungfer K.H. (2011) Description of the tadpoles of *Hypsiboas aguilari* and *H. melanopleura* (Anura: Hylidae: *Hypsiboas pulchellus* group). Salamandra 47: 30-35.

Lourenço L.B., Garcia P.C.A., Recco-Pimentel S.M. (2003) Cytogenetics of a new species of *Paratelmatobius cardosoi* group (Anura: Leptodactylidae), with the descriptionof an apparent case of pericentric inversion. Amphibia-Reptilia 24: 47-5.

Martirosyan A., Stepanyan I. (2007) The karyotype of *Hyla savignyi* Audouin, 1827 (Amphibia: Anura) from Southern Armenia. Comp. Cytog. 1(2): 107-112.

Martori R., Ávila L.J. (1992) Confirmación de la Presencia de *Hyla pulchella pulchella* para la Provincia de Córdoba (Argentina). Bol. Asoc. Herp. Arg. 8: 4-5.

Morescalchi A. (1973) Amphibia. In: Chiarelli A.B, Campanna E. (Eds.) Cytotaxonomy and Vertebrate Evolution. Academics Press, London, United Kingdom, pp. 233-348.

Raber S.C., Carvalho K.A., Garcia P.C.A., Vinciprova G., Recco-Pimentel S.M. (2004) Chromosomal characterization of *Hyla bischoffi* and *Hyla guentheri* (Anura, Hylidae). Phyllomedusa 3: 43-49.

Reyes Valdés M.H., Martínez M.G., García Osuna H.T. (2000) Número cromosómico y apareamiento meiótico en *Turbinicarpus valdezianus* (Möller) Glass & Foster (Cactaceae). Acta Bot. Mex. 53: 17-25.

Salas N.E. (2006) Análisis cromosómico de *Odontophrynus americanus*, *O. achalensis*, *O. cordobae* y *O. occidentalis* (Anura: Leptodactylidae) de la provincia de Córdoba, Argentina. Rev. Esp. Herp. 20: 31-38.

Salas N.E., Martino A.L. (2007) Cariotipo de *Odontophrynus cordobae* Martino & Sinsch, 2002 (Anura Leptodactylidae). BAG, J. Basic Appl. Genet. 18: 1-5.

Schmid M. (1978) Chromosome banding in Amphibia I. Constitutive heterochromatin and nucleolus organizer regions in *Bufo* and *Hyla*. Chromosoma 66: 361-388.

Schmid M., Olert J., Klett C. (1979) Chromosome banding in Amphibia III. Sex chromosomes in *Triturus*. Chromosoma 71: 29-55.

Schmid M., Steinlein C., Nanda I., Epplen J.T. (1990) Chromosome banding in Amphibia. In: Olmo E. (Ed.) Cytogenetics of Amphibians and Reptiles. Birkhäuser Verlag Berlin, pp. 21-45.

Stuart S. (2006) *Hypsiboas cordobae*. IUCN Red List de Threatened Species. Version 2009.1. http//www.iucnredlist.org/ (accessed June 2009).

Sumner A.T. (1972) A simple technique for demonstrating centromeric heterochromatin. Exp. Cell. Res. 75: 304–306.

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