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Intrageneric Karyotypic Variation in *Pseudopaludicola* (Anura: Leiuperidae) and Its Taxonomic Relatedness

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Eduardo R. Fávero, Ana C. P. Veiga-Menoncello, Denise C. Rossa-Feres, Christine Strüssmann, Ariovaldo A. Giaretta, Gilda V. Andrade, Patrick Colombo, and Shirlei M. Recco-Pimentel (2011) Intrageneric karyotypic variation in *Pseudopaludicola* (Anura: Leiuperidae) and its taxonomic relatedness. *Zoological Studies* **50**(6): 826-836. Herein, we report karyological data for *Pseudopaludicola* frogs, with the main objective to investigate the confusing taxonomic identification of these anurans. The samples analyzed included topotypes of *P. falcipes* and *P. ameghini* (currently considered a synonym of *P. mystacalis*) in addition to specimens of *P. mystacalis* and *P. ternetzi* and of 8 additional populations of *Pseudopaludicola* from several regions of Brazil. Interspecific variations in the chromosome number, location of the nucleolar organizer region (NOR), and banding patterns were observed. The karyotype of *P. falcipes* consisted of 2*n* = 22 chromosomes, whereas *P. ameghini* (sensu Cope 1887) and *P. ternetzi* had 2*n* = 20 and *P. mystacalis* had 2*n* = 16 chromosomes. The chromosome number 2*n* = 16 was also found in specimens of populations from Santa Fé do Sul, Vitória Brazil, Icém, Palestina (southeastern Brazil), Barreirinhas, Urbano Santos (northeastern Brazil), Poconé, and Santa Terezinha (west-central Brazil). Nevertheless, in some individuals from Icém and Poconé, we found 2*n* = 20 and 2*n* = 22 chromosomes, respectively. This variation in chromosome number indicates diverse taxa coexisting in sympatry. The karyotypic patterns of 2*n* = 20 in *P. ameghini* (sensu Cope 1887) and 2*n* = 16 in *P. mystacalis* are clearly indicative of 2 distinct taxonomic units. Therefore, our data corroborate the removal of *P. ameghini* from synonymy with *P. mystacalis* as previously suggested. We define the karyotype of *P. falcipes* as 2*n* = 22 and of *P. ternetzi* as 2*n* = 20 and reinforce the need for a taxonomic revision of this genus.
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Key words: *Pseudopaludicola*, Cytotaxonomy, Karyotype, Ag-NOR, C-band.

The genus *Pseudopaludicola* Miranda Ribeiro (1926) belongs to the family Leiuperidae (Frost 2011) and contains anurans measuring up to 20 mm. Systematics assigns individuals that are very similar to each other to distinct species (Haddad and Cardoso 1987). Representatives of this genus are widespread throughout South America, from Colombia to Argentina, including the Guyanas and Suriname (Lobo 1992). The genus *Pseudopaludicola* consists of 13 species:

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P. boliviana, *P. canga*, *P. ceratophryes*, *P. falcipes*, *P. llanera*, *P. mineira*, *P. murundu*, *P. mystacalis*, *P. pusilla*, *P. riopiedadensis*, *P. saltica*, *P. serrana*, and *P. ternetzi* (Frost 2011); eight of them are found in Brazil.

The taxonomic structure of the genus *Pseudopaludicola* historically has undergone various changes. Milstead (1963) considered the species *P. ameghini*, *P. mystacalis*, and *P. saltica* to be synonyms of *P. falcipes*. However, Bokermann (1966) recognized these 4 species in western and southeastern Brazil and synonymized *P. ternetzi* with *P. ameghini*. Lynch (1971); however, considered 5 species to be valid, *P. ameghini*, *P. falcipes*, *P. mystacalis*, *P. saltica*, and *P. ternetzi*, without providing evidence to support withdrawing *P. ternetzi* from the *P. ameghini* synonymy. Afterwards, Haddad and Cardoso (1987) performed bioacoustic and morphometric analyses of the topotypes of *P. saltica*, *P. mystacalis*, and *P. ameghini*, all described by Cope (1887) from Chapada dos Guimarães, Mato Grosso, Brazil. Because the authors found only *P. saltica* and *P. mystacalis* in sympatry, they considered both to be valid species for that locality and *P. ameghini* to be synonymous with *P. mystacalis*. After examining the types, Lobo (1996) corroborated this taxonomic change and validated the species *P. mystacalis* and *P. ternetzi*. Recently, revalidation of *P. ameghini* (sensu Cope 1887) was proposed by Strüssmann (2003) based on additional morphometric and bioacoustic analyses and on the recognition of 3 distinct taxonomic units in the area once occupied by the huge municipality of Chapada dos Guimarães.

Altogether, there is little cytogenetic information on the genus *Pseudopaludicola*, and it is mostly restricted to karyotype analyses by conventional Giemsa staining. Moreover, published data are conflicting. For instance, Beçak (1968) described the *P. falcipes* karyotype with 2*n* = 18 and the *P. ameghini* karyotype with 2*n* = 20 chromosomes in specimens from the municipality of São José do Rio Preto, São Paulo, Brazil. However, Batistic et al. (1969 1970) and Batistic (1970) reported distinct karyotypic configurations for populations treated as *P. falcipes* sampled in distinct regions of Brazil. The "*P. falcipes*" species varied from 2*n* = 16 to 2*n* = 20 in São Paulo and Minas Gerais states and was characterized as 2*n* = 16 and 2*n* = 20 in populations from the state of Mato Grosso and *n* = 11 in meiotic cells of a population from the state of Bahia. Studies of *P. falcipe*s from the southernmost region of South America, near its type locality, revealed only populations with 22 chromosomes (Saez and Brum 1960, Brum-Zorrilla and Saez 1968). Nevertheless, the available cytogenetic data have proven to be useful for distinguishing morphologically cryptic or similar *Pseudopaludicola* species (Medeiros et al. 2003, Veiga-Menoncello et al. 2006).

In the present work, topotypes of *P. falcipes* and *P. ameghini* (sensu Cope 1887), populations of *P. ternetzi* and *P. mystacalis*, and specimens from 8 additional populations sampled in several regions of Brazil, comprising *Pseudopaludicola* sp. (aff. *falcipes*), *Pseudopaludicola* sp. (aff. *mystacalis*), *Pseudopaludicola* cf. *ternetzi*, and *Pseudopaludicola* sp., were cytogenetically analyzed. The objectives were to investigate the confusing taxonomic identification and the wide intrageneric chromosome number variation, with emphasis on *P. falcipes*, and contribute to a better understanding of *Pseudopaludicola* systematics.

MATERIAL AND METHODS

Specimen sampling

Pseudopaludicola specimens were sampled in 13 locations of Brazil, as shown in figure 1. The studied populations, sampling locations, and identification of voucher specimens deposited in museums are listed in table 1. All specimens were collected under authorization of the Instituto Brasileiro do Meio Ambiente e de Recursos Naturais Renováveis (IBAMA, with license no. 02001.002003/2005-16).

Chromosomal preparation

Chromosomes were prepared from suspensions of intestinal epithelial and testis cells from animals previously treated with 2% colchicine for at least 5 h, according to King and Rofe (1976) and Schmid (1978). Metaphases were stained with 10% Giemsa in pH 6.8 phosphate buffer for 10 min, or with silver (Ag-NOR) according to Howell and Black (1980). They were C-banded according to Sumner (1972) with the following modification. Slides were pretreated in 50% acetic acid for 30 min (Siqueira et al. 2008).

RESULTS

Cytogenetic data for *Pseudopaludicola* frog species analyzed are presented in table 2. The species and populations were grouped according to their chromosome number and comprised the topotypes of *P. falcipes*, *P. ameghini* (sensu Cope 1887), and *P. mystacalis* plus specimens of *P. ternetzi* and of populations from 8 localities. The latter specimens were named *Pseudopaludicola* sp. (aff. *falcipes*), *Pseudopaludicola* sp. (aff. *mystacalis*), and *Pseudopaludicola* sp. according to their external morphological features, as shown in table 1.

Pseudopaludicola falcipes and *Pseudopaludicola* sp. 1

The karyotypes contained 11 pairs (2*n* = 22) of highly similar chromosomes. Pairs 1-3, 5, 6, and 9-11 were metacentric, pairs 4 and 7 were submetacentric, and pair 8 was subtelocentric (Figs. 2A, 3A). In *P. falcipes*, a nucleolar organizing region (NOR) was localized in the pericentromeric region of the long arm of pair 8 (Fig. 2A), whereas in *Pseudopaludicola* sp. 1 the NOR was in the interstitial region of the long arm of pair 8 (Fig. 3A). In all *P. falcipes* specimens, the NOR size was heteromorphic in homologous chromosomes

Fig. 1. Map of Brazil displaying the sampling locations in which specimens of the genus *Pseudopaludicola* were surveyed. **1.** Porto Alegre - RS. **2.** Chapada dos Guimarães - MT. **3.** Vila Bela da Santíssima Trindade – MT. **4.** Cuiabá – MT. **5.** Uberlândia - MG. **6.** Santa Fé do Sul - SP. **7.** Vitória Brasil SP. **8.** Icém - SP. **9.** Palestina - SP. **10.** Barreirinhas - MA. **11.** Urbano Santos - MA. **12.** Poconé - MT. **13.** Santa Teresinha - MT.

Table 1. *Pseudopaludicola* species, sampling locations in Brazil, number of analyzed individuals, and identification of voucher specimens in the Museu de Zoologia "Prof. Adão José Cardoso" (ZUEC), Instituto de Biologia, Univ. Estadual de Campinas, SP and in the "Coleção Amphibia" (DZSJRP), Univ. Estadual Paulista (UNESP), São José do Rio Preto, SP. [a]Two distinct karyotypes were identified in *Pseudopaludicola* sp. from Poconé (MT)

Sampled taxa	Sampling locations	Number of specimens	Voucher specimens in museums
P. falcipes - topotype	Porto Alegre (Rio Grande do Sul State) (30°00'59"S, 51°06'07"W)	4 ♂♂, 2 ♀♀	ZUEC: 14008, 14009, 14022, 14023, 14162, 14163
P. ameghini (sensu Cope 1887) - topotype	Chapada dos Guimarães (Mato Grosso State) (15°27'38"S, 55°44'59"W)	2 ♂♂, 1 ♀	ZUEC: 14138, 14139, 14140
P. ameghini (sensu Cope 1887)	Vila Bela da Santíssima Trindade (Mato Grosso State) (15°00'29"S, 59°57'02"W)	5 ♂♂	ZUEC: 13923, 13924, 13925, 13926, 14146
P. mystacalis	Cuiabá (Mato Grosso State) (15°35'46"S, 56°05'48"W)	5 ♂♂, 1 ♀	ZUEC: 14149, 14150, 14141, 14142, 14153, 14157
P. mystacalis	Uberlândia (Minas Gerais State) (18°55'07"S, 48°16'38"W)	6 ♂♂, 2 ♀♀	ZUEC: 14128, 13876, 13878, 13879, 13880, 13881, 13882, 13883
Pseudopaludicola sp. (aff. *falcipes*)	Santa Fé do Sul (São Paulo State) (20°12'40"S, 50°55'33"W)	5 ♂♂	DZSJRP: 8694, 8695, 8696, 8705, 8710
Pseudopaludicola sp. (aff. *falcipes*)	Vitória Brasil (São Paulo State) (20°11'48"S, 50°29'04"W)	4 ♂♂, 6 ♀♀	ZUEC: 13894, 13895, 13896, 13898, 13900, 13904, 13905, 13906, 13907, 13908
Pseudopaludicola sp. (aff. *falcipes*)	Icém (São Paulo State) (20°20'30"S, 49°11'42"W)	4 ♂♂, 1 ♀	DZSJRP: 6438, 6441, 6448, 6452, 8720
Pseudopaludicola sp. (aff. *falcipes*)	Palestina (São Paulo State) (20°23'24"S, 49°25'59"W)	1 ♂	ZUEC: 13912
Pseudopaludicola cf. *ternetzi*	Icém (São Paulo State) (20°20'30"S, 49°11'42"W)	2 ♂♂	DZSJRP: 6456, 6457
Pseudopaludicola sp. (aff. *mystacalis*) I	Icém (São Paulo State) (20°20'30"S, 49°11'42"W)	2 ♂♂	DZSJRP: 8723, 8724
Pseudopaludicola sp. (aff. *mystacalis*) II	Barreirinhas (Maranhão State) (2°38'11"S, 42°41'26"W)	4 ♂♂, 2 ♀♀	ZUEC: 13842, 13843, 13844, 13845, 13846, 13849
Pseudopaludicola sp. (aff. *mystacalis*) II	Urbano Santos (Maranhão State) (3°12'28"S, 43°09'12"W)	4 ♂♂, 2 ♀♀	ZUEC: 13868, 13869, 13870, 13871, 13916, 13915
P. ternetzi	Uberlândia (Minas Gerais State) (18°55'07"S, 48°16'38"W)	2 ♂♂, 1 ♀	ZUEC: 14170, 14171, 14172
Pseudopaludicola sp. 1	[a]Poconé (Mato Grosso State) (16°15'24"S, 56°37'22"W)	4 ♂♂, 2 ♀♀	ZUEC: 13929, 13930, 13931, 13932, 13933, 13934
Pseudopaludicola sp. 2	[a]Poconé (Mato Grosso State) (16°15'24"S, 56°37'22"W)	2 ♂♂, 1 ♀	ZUEC: 13935, 13936, 13937
Pseudopaludicola sp. 3	Santa Terezinha (Mato Grosso State) (10°28'11"S, 50°30'11"W)	1 ♂	ZUEC: 13805

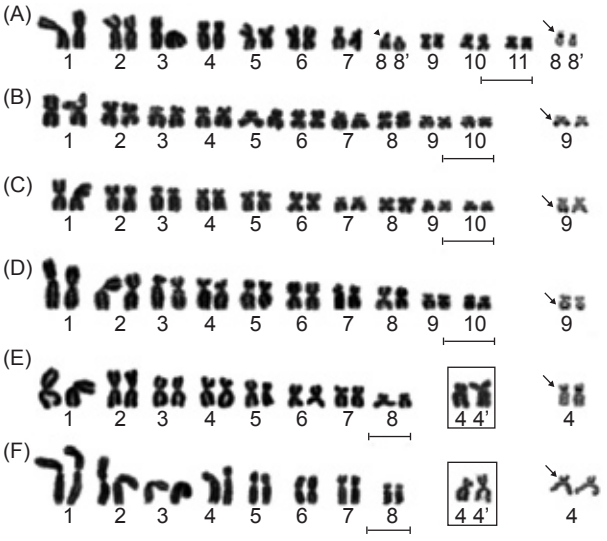

Fig. 2. Karyotypes stained with Giemsa and silver. (A) *Pseudopaludicola falcipes* from Porto Alegre (Rio Grande do Sul State); (B) *P. ameghini* (sensu Cope 1887) from Chapada dos Guimarães (Mato Grosso State); (C) *P. ameghini* (sensu Cope 1887) from Vila Bela da Santíssima Trindade (Mato Grosso State); (D) *P. ternetzi* from Uberlândia (Minas Gerais State); (E) *P. mystacalis* from Cuiabá (Mato Grosso State); (F) *P. mystacalis* from Uberlândia (Minas Gerais State). The arrow indicates pair 8, heteromorphic in *P. falcipes*, with a conspicuous secondary constriction. Insets show the heteromorphic pair 4 in *P. mystacalis* (4 4'). Arrows indicate the nucleolar organizing region (NOR). Note the heteromorphic NOR in *P. falcipes*. In the insets, note pair 4 of *P. mystacalis* with size heteromorphism (4 4'). Scale bar = 5 μm.

of pair 8, whereas in *Pseudopaludicola* sp. 1 the NOR was homomorphic. Heterochromatic blocks were present in all centromeres of both species. In *P. falcipes*, pericentromeric regions of pair 3 contained heterochromatic blocks, strongly marked in the short arm and more weakly in the long arm. In addition, heterochromatic bands were observed in the pericentromeric region of the long arm of pair 8, coincident with the NOR site. As for *Pseudopaludicola* sp. 1, heterochromatic blocks were detected in pericentromeric regions of the short arms of pairs 1, 7, and 9 and in the long arm of pair 2. Pair 8 showed a C-band in the interstitial region, adjacent to the NOR (Figs. 4A, 5A).

Pseudopaludicola ameghini (sensu Cope 1887), *P. ternetzi*, and *Pseudopaludicola* cf. *ternetzi*

The karyotypes of these species consisted of 2*n* =20 chromosomes. In all *P. ameghini* specimens, which were sampled in 2 locations of Mato Grosso State (the type locality at Chapada dos Guimarães and Vila Bela da Santíssima Trindade), the karyotype consisted of metacentric (pairs 1, 2, 6, and 8), submetacentric (pairs 3-5, 9, and 10), and subtelocentric chromosomes (pair 7). In *P. ternetzi*, a major difference was observed in the morphology of pair 7, which was classified as submetacentric (Fig. 2B-D). The karyotypes of a

Table 2. Chromosomal morphometry and centromeric classification in representatives of the genus *Pseudopaludicola* from several Brazilian localities, according to values proposed by Green and Sessions (1991). [a]Indicates the nucleolar organizing region (NOR)-bearing chromosome pair and its arm. RS, relative size; AR, arm ratio; CC, chromosome classification; M, metacentric; SM, submetacentric; ST, subtelocentric; p, short arm; q: long arm.

	Chromosomes												
	1	2	3	4	4'	5	6	7	8	8'	9	10	11
Pseudopaludicola falcipes - Porto Alegre (Rio Grande do Sul State)													
RS	15.07	12.85	12.48	11.11	-	9.58	8.339	7.922	6.464	5.274	6.236	5.222	4.689
AR	1.204	1.170	1.476	2.100		1.13	1.069	2.111	3.674	3.612	1.050	1.099	1.205
CC	M	M	M	SM		M	M	SM	[a]ST(q)	[a]ST(q)	M	M	M
Pseudopaludicola sp. 1 - Poconé (Mato Grosso State)													
RS	15.11	13.27	11.17	10.59	-	9.875	8.919	8.346	7.499	-	5.809	5.198	4.210
AR	1.080	1.136	1.558	1.978		1.197	1.110	1.675	3.732	-	1.273	1.310	1.477
CC	M	M	M	SM		M	M	SM	[a]ST(q)	-	M	M	M
Pseudopaludicola ameghini (sensu Cope 1887) - Chapada dos Guimarães (Mato Grosso State)													
RS	16.79	13.32	11.76	10.60	-	9.621	9.523	8.966	8.897	-	5.723	4.776	
AR	1.032	1.097	2.106	2.142		2.243	1.144	3.869	1.155	-	2.209	2.178	
CC	M	M	SM	SM		SM	M	ST	M	-	[a]SM(q)	SM	
Pseudopaludicola ameghini (sensu Cope 1887) - Vila Bela da Santíssima Trindade (Mato Grosso State)													
RS	17.16	13.80	12.94	11.11	-	10.01	9.281	7.949	7.936	-	5.412	4.386	

Table 2. (Continued)

	Chromosomes												
	1	2	3	4	4'	5	6	7	8	8'	9	10	11
AR	1.184	1.219	2.771	2.361		2.161	1.115	4.178	1.102	-	2.356	2.439	
CC	M	M	SM	SM		SM	M	ST	M	-	[a]SM(q)	SM	
Pseudopaludicola ternetzi - Uberlândia (Minas Gerais State)													
RS	16.43	13.70	11.63	10.84	-	10.26	9.434	8.842	7.888	-	5.933	5.028	
AR	1.152	1.163	2.333	2.230		2.244	1.027	2.044	1.033	-	1.967	2.000	
CC	M	M	SM	SM		SM	M	SM	M	-	[a]SM(q)	SM	
Pseudopaludicola cf. *ternetzi* - Icém (São Paulo State)													
RS	15.807	13.97	11.98	10.48	-	10.31	10.31	9.650	8.319	-	4.991	4.159	
AR	1.1591	1.2105	2.272	2.150		1.818	1.214	1.071	1.083		2.000	2.125	
CC	M	M	SM	SM		SM	M	M	M		SM	SM	
Pseudopaludicola mystacalis - Cuiabá (Mato Grosso State)													
RS	21.79	17.13	13.71	11.55	13.07	10.19	10.01	9.241	6.336				
AR	1.057	1.054	1.473	2.134	1.634	1.900	1.120	1.974	2.336				
CC	M	M	M	[a]SM(p)	[a]M(p)	SM	M	SM	SM				
Pseudopaludicola mystacalis - Uberlândia (Minas Gerais State)													
RS	21.20	17.34	13.44	11.79	13.50	10.32	9.897	9.539	6.451				
AR	1.061	1.065	1.522	2.333	1.646	2.023	1.065	2.115	2.037				
CC	M	M	M	[a]SM(p)	[a]M(p)	SM	M	SM	SM				
Pseudopaludicola sp. (aff. *mystacalis*) I - Icém (São Paulo State)													
RS	20.5	16.89	13.24	12.19	13.76	10.45	10.36	9.669	6.620				
AR	1.033	1.088	1.404	2.050	1.566	1.930	1.095	1.983	2.069				
CC	M	M	M	[a]SM(p)	[a]M(p)	SM	M	SM	SM				
Pseudopaludicola sp. (aff. *mystacalis*) II - Barreirinhas (Maranhão State)													
RS	20.85	16.81	13.54	12.33	13.74	10.24	9.894	9.389	6.924				
AR	1.115	1.089	1.392	2.053	1.794	1.827	1.116	1.932	1.898				
CC	M	M	M	[a]SM(p)	[a]SM(p)	SM	M	SM	SM				
Pseudopaludicola sp. (aff. *mystacalis*) II - Urbano Santos (Maranhão State)													
RS	20.73	16.51	13.92	12.41	13.66	10.43	9.646	9.419	6.916				
AR	1.096	1.055	1.284	1.694	1.651	1.897	1.114	1.827	2.160				
CC	M	M	M	[a]SM(p)	[a]M(p)	SM	M	SM	SM				
Pseudopaludicola sp. (aff. *falcipes*) - Santa Fé do Sul (São Paulo State)													
RS	21.70	17.73	13.60	11.89	13.24	10.06	9.650	8.809	6.528				
AR	1.075	1.083	1.375	2.213	1.845	1.977	1.090	2.017	2.042				
CC	M	M	M	[a]SM(p)	[a]SM(p)	SM	M	SM	SM				
Pseudopaludicola sp. (aff. *falcipes*) - Vitória Brasil (São Paulo State)													
RS	20.65	17.71	13.85	12.48	13.49	10.35	9.617	9.076	6.231				
AR	1.068	1.096	1.405	2.214	1.894	1.914	1.129	1.950	2.103				
CC	M	M	M	[a]SM(p)	[a]SM(p)	SM	M	SM	SM				
Pseudopaludicola sp. (aff. *falcipes*) - Icém (São Paulo State)													
RS	20.73	17.12	13.91	13.10	14.13	10.34	9.356	9.048	6.377				
AR	1.104	1.090	1.405	2.041	1.586	1.946	1.242	1.843	2.069				
CC	M	M	M	[a]SM(p)	[a]M(p)	SM	M	SM	SM				
Pseudopaludicola sp. (aff. *falcipes*) - Palestina (São Paulo State)													
RS	22.59	16.61	13.74	12.22	13.57	10.70	9.180	8.490	6.453				
AR	1.375	1.227	1.382	2.000	1.857	2.150	1.076	2.125	2.166				
CC	M	M	M	[a]SM(p)	[a]SM(p)	SM	M	SM	SM				
Pseudopaludicola sp. 2 - Poconé (Mato Grosso State)													
RS	21.35	17.11	14.32	11.63	13.35	10.12	9.934	8.826	6.684				
AR	1.070	1.065	1.370	2.00	1.682	2.036	1.075	1.920	2.171				
CC	M	M	M	[a]SM(p)	[a]SM(p)	SM	M	SM	SM				
Pseudopaludicola sp. 3 - Santa Terezinha (Mato Grosso State)													
RS	20.60	17.38	14.51	12.77	13.67	9.869	9.305	8.513	7.026				
AR	1.025	1.084	1.451	1.979	1.644	2.125	1.078	1.939	2.018				
CC	M	M	M	[a]SM(p)	[a]M(p)	SM	M	SM	SM				

few specimens from Icém (São Paulo), designated here as *Pseudopaludicola* cf. *ternetzi*, were similar to those of *P. ameghini* (sensu Cope 1887) and *P. ternetzi* except for pair 7, which was classified as being metacentric (Fig. 3B). In *P. ameghini* and *P. ternetzi*, a NOR site was identified in the telomeric region of the long arm of chromosome 9 (Fig. 2B-D), and centromeric bands were detected in all chromosomes. In addition, pericentromeric bands were observed in the short arms of pairs 1 and 2 of *P. ameghini* (sensu Cope 1887) and *P. ternetzi*. These species could be distinguished by the presence of pericentromeric bands in the long arms of pairs 3, 4, and 5 in *P. ameghini* (sensu Cope 1887), whereas in *P. ternetzi*, they were in the long arms of pairs 1, 2, and 3 and in the telomeric region of pair 4 (Fig. 4B-D). Specimens of *Pseudopaludicola* cf. *ternetzi* were not analyzed by C-banding or silver staining.

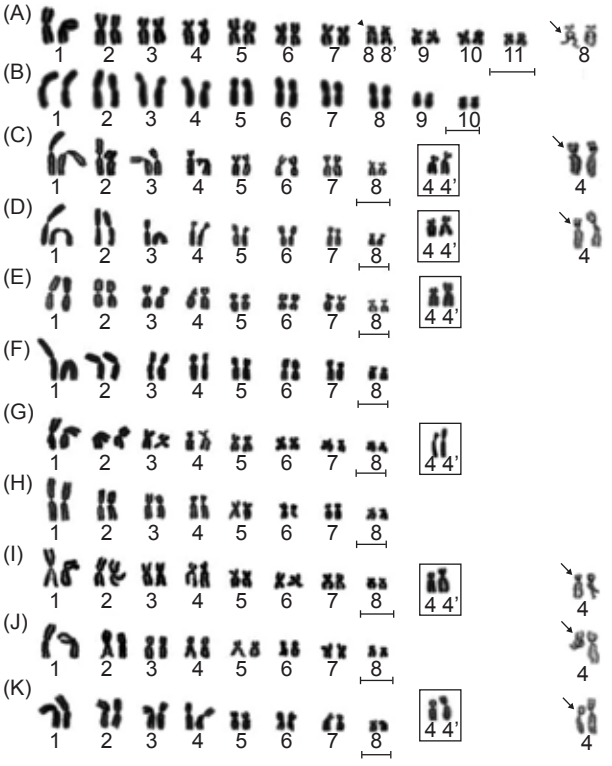

Fig. 3. Karyotypes stained with Giemsa and silver. (A) *Pseudopaludicola* sp. 1 from Poconé (Mato Grosso State); (B) *Pseudopaludicola* cf. *ternetzi* from Icém (São Paulo State); (C) *Pseudopaludicola* sp. (aff. *falcipes*) from Santa Fé do Sul (São Paulo State); (D) *Pseudopaludicola* sp. (aff. *falcipes*) from Vitória Brasil (São Paulo State); (E) *Pseudopaludicola* sp. (aff. *falcipes*) from Icém (São Paulo State); (F) *Pseudopaludicola* sp. (aff. *falcipes*) from Palestina (São Paulo State); (G) *Pseudopaludicola* sp. (aff. *mystacalis*) I from Icém (São Paulo State); (H) *Pseudopaludicola* sp. (aff. *mystacalis*) II from Barreirinhas (Maranhão State); (I) *Pseudopaludicola* sp. (aff. *mystacalis*) II from Urbano Santos (Maranhão State); (J) *Pseudopaludicola* sp. 2 from Poconé (Mato Grosso State); (K) *Pseudopaludicola* sp. 3 from Sta. Terezinha (Mato Grosso State). The arrowhead in *Pseudopaludicola* sp. 1 indicates the homomorphic pair 8 with a conspicuous secondary constriction. Arrows indicate the nucleolar organizing region (NOR). The insets show the heteromorphic pair 4 (4 4') in *Pseudopaludicola* sp. (aff. *falcipes*) from Santa Fé do Sul (SP). Scale bar = 5 μm.

Pseudopaludicola mystacalis, *Pseudopaludicola* spp. (aff. *mystacalis*) I and II, *Pseudopaludicola* sp. (aff. *falcipes*), and *Pseudopaludicola* spp. 2 and 3

Karyotypes of *P. mystacalis* and specimens of the populations mentioned above consisted of 2*n* = 16 and were comprised of metacentric (1-3 and 6) and submetacentric (4, 5, 7, and 8) chromosome pairs (Figs. 2E, F, 3C-K). However,

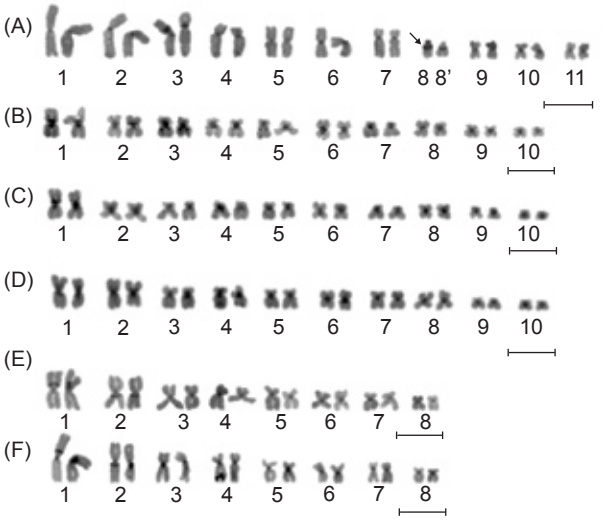

Fig. 4. Karyotypes stained by the C-banding technique. (A) *Pseudopaludicola* falcipes from Porto Alegre (Rio Grande do Sul State); (B) *P. ameghini* (sensu Cope 1887) from Chapada dos Guimarães (Mato Grosso State); (C) *P. ameghini* (sensu Cope 1887) from Vila Bela da Santíssima Trindade (Mato Grosso State); (D) *P. ternetzi* from Uberlândia (Minas Gerais State); (E) *P. mystacalis* from Cuiabá (Mato Grosso State); (F) *P. mystacalis* from Uberlândia (Minas Gerais State). The arrow in *P. falcipes* indicates pair 8 with a heteromorphic heterochromatic area in the pericentromeric region. Scale bar = 5 μm.

in a few specimens of these populations and of *P. mystacalis*, pair 4 was heteromorphic. The smaller morph was classified as submetacentric and the larger one as metacentric (Figs. 2E, F, 3C-E, G, I, K). This characteristic was not sex-related. A NOR was localized in the pericentromeric region of the short arm of pair 4 in all specimens analyzed (Figs. 2E, F, 3C, D, I-K) including specimens from Icém, Palestina (São Paulo) and Barreirinhas (Maranhão) (data not shown). Besides centromeric C-bands in all chromosomes, pericentromeric blocks were identified in the short arm of pair 1 and in the long arm of pair 2 of all specimens of the populations studied (Figs. 4E, F, 5B-F). All specimens of *Pseudopaludicola* sp. (aff. *falcipes*) from Vitória Brasil (São Paulo) and of *Pseudopaludicola* sp. 3 from Santa Terezinha (Mato Grosso) that had the heteromorphic pair 4 exhibited interstitial heteromorphic bands in the short arm of pair 4 (Fig. 5B, F). Silver staining and C-banding techniques were not used on the sample from Icém (São Paulo), named *Pseudopaludicola* sp. (aff. *mystacalis*) I, due to a lack of biological material.

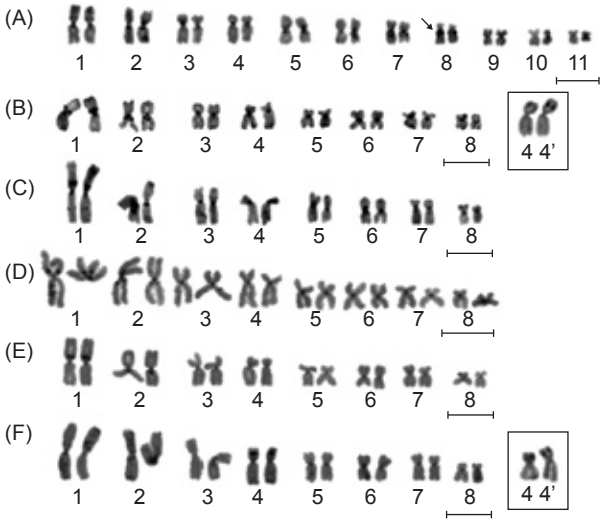

Fig. 5. Karyotypes stained by the C-banding technique. (A) *Pseudopaludicola* sp. 1 from Poconé (Mato Grosso State); (B) *Pseudopaludicola* sp. (aff. *falcipes*) from the northwestern region of São Paulo State (Santa Fé do Sul, Vitória Brasil, Icém, and Palestina); (C) *Pseudopaludicola* sp. (aff. *mystacalis*) II from Barreirinhas (Maranhão State); (D) *Pseudopaludicola* sp. (aff. *mystacalis*) II from Urbano Santos (Maranhão State); (E) *Pseudopaludicola* sp. 2 from Poconé (Mato Grosso State); (F) *Pseudopaludicola* sp. 3 from Sta. Terezinha (Mato Grosso State). In *Pseudopaludicola* sp. 1, the arrow indicates pair 8 with heterochromatic homomorphic regions. The insets of *Pseudopaludicola* sp. (aff. *falcipes*) from Vitória Brasil and *Pseudopaludicola* sp. 3 from Poconé show a heteromorphic C-band in the interstitial region of the short arm of pair 4. Scale bar = 5 μm.

DISCUSSION

The data revealed interspecific variations in chromosome number, NOR localization, and patterns of heterochromatin distribution among the *Pseudopaludicola* species and populations analyzed. The karyotypic polymorphisms identified were suitable for distinguishing the species studied and represent substantial traits for taxonomic studies. Data in the literature on chromosomes of *Pseudopaludicola* species have been conflicting and scarce. Of the 13 species currently valid for this genus (Frost 2011), only a few populations of *P. falcipes* and *P. mystacalis* (in reality, *P. ameghini* sensu Cope 1887) have been analyzed cytogenetically (Saez and Brum 1960, Beçak 1968, Brum-Zorrilla and Saez 1968, Batistic et al. 1969 1970, Batistic 1970).

The karyotype of 2*n* = 22 chromosomes in *P. falcipes* sampled in its type locality, Porto Alegre, Rio Grande do Sul State, differed from data previously reported by Beçak (1968), who described a diploid complement of 18 chromosomes. The data of this report also disagree with Batistic et al. (1969 1970) and Batistic (1970), who reported an interpopulational variation attributed to *P. falcipes* with karyotypes consisting of 2*n* = 16, 18, and 20 chromosomes in specimens sampled near São José do Rio Preto City, in the northwestern region of São Paulo State, and from several localities in the states of Mato Grosso and Minas Gerais. Batistic (1970), however, observed the presence of 11 bivalents (2*n* = 22) in meiotic cells of males, putatively *P. falcipes*, from Feira de Santana (Bahia).

Our results partially agree with those described for *P. falcipes* populations from Uruguay (Saez and Brum 1960, Brum-Zorrilla and Saez 1968). Despite having equal chromosome numbers, those Uruguayan populations and *P. falcipes* from the type locality differ in their chromosomal morphology. Saez and Brum (1960) described the presence of only metacentric and submetacentric chromosomes, while our results revealed the existence of metacentric and submetacentric pairs and 1 subtelocentric pair. This karyotype polymorphism suggests a possible misinterpretation in *P. falcipes* identification in Uruguayan populations, because species of the genus *Pseudopaludicola* typically have similar external morphologies, and some of them are syntopic. Moreover, the presence of 2*n* = 22 in different populations could indicate the existence of more than 1 *Pseudopaludicola* species that

shares this number of chromosomes.

Karyotypic comparisons of *P. falcipes* from its type locality with the population identified as *P. falcipes* (*n* = 11) from the state of Bahia (Batistic 1970) were not possible because only meiotic cells were analyzed in the latter case. The specimens denoted *Pseudopaludicola* sp. 1, from Poconé (Mato Grosso) also showed a diploid number of 22 chromosomes, and the karyotype was very similar to the *P. falcipes* topotype. These results suggest proximity between these taxa. Further studies on both populations, including comparative analyses of the external morphology and vocalization patterns, are necessary to support the hypothesis of a wide geographic distribution of *P. falcipes* and related species, because *P. falcipes* was originally described from southern Brazil (State of Rio Grande do Sul), and currently its distribution is still recognized as southern Brazil through southeastern Paraguay and Uruguay to northeastern Argentina (Frost 2011).

Outstanding differences in chromosome numbers and NOR localizations described in this report clearly differentiate the type locality specimens of *P. falcipes* (2*n* = 22) from *P. ameghini* (sensu Cope 1887) (2*n* = 20), *P. ternetzi* (2*n* = 20), and *P. mystacalis* (2*n* = 16), as well as from populations named *Pseudopaludicola* sp. (aff. *falcipes*) (2*n* = 16), *Pseudopaludicola* cf. *ternetzi* (2*n* = 20), and *Pseudopaludicola* spp. (aff. *mystacalis*) I and II (2*n* = 16). Therefore, the results corroborate the previous proposal to remove *P. ameghini* from synonymy with *P. mystacalis* (Strüssmann 2003). The *P. ameghini* (sensu Cope 1887) karyotype contains 2*n* = 20 chromosomes and differs from *P. mystacalis* in chromosome number and morphology, heterochromatin distribution, and position of the NOR.

The karyotypic data did not distinguish specimens of populations of *Pseudopaludicola* sp. (aff. *falcipes*) from *Pseudopaludicola* spp. (aff. *mystacalis*) I and II or *Pseudopaludicola* spp. 2 and 3. Despite having the same number of chromosomes, populations of *Pseudopaludicola* sp. (aff. *falcipes*) and those of *Pseudopaludicola* sp. (aff. *mystacalis*) I from the northwestern region of São Paulo State can be distinguished by several morphological features such as the width of the head, the shape of the snout, and the size of the metatarsal tubercles (D.C. Rossa-Feres, personal information). All these populations differ morphologically from *P. falcipes* from southern Brazil (State of Rio Grande do Sul). Specimens studied by Batistic et al. (1969 1970) and Batistic (1970) certainly did not represent different populations of *P. falcipes*, because karyotypes with 2*n* = 22 were not found in northwestern São Paulo State. Therefore, our results indicate a geographic distribution for *P. falcipes* populations that excludes the northwestern region of the state of São Paulo.

Furthermore, a karyotype with 2*n* = 20 chromosomes, similar to *P. ameghini* (sensu Cope 1887) and *P. ternetzi*, was found in specimens from Icém (São Paulo) named *Pseudopaludicola* cf. *ternetzi*. The small number of analyzed specimens, slight morphological differences in pair 7, and the lack of information on C-bands and NOR localization do not allow an unequivocal discrimination of this population from the others that share the same number of chromosomes.

In Icém, São Paulo State, some of the specimens named *Pseudopaludicola* sp. (aff. *canga*) were found to have karyotypes with 2*n* = 18 chromosomes, similar to *P. canga* (Duarte et al. 2010). Thus, in northwestern São Paulo State, 2 morphological forms with 2*n* = 16, and species with 2*n* = 18 and 2*n* = 20 chromosomes were found in sympatry. These observations indicate the necessity of a taxonomic review that may result in the identification of presently unrecognized representatives of this genus in northwestern São Paulo State.

Specimens named *Pseudopaludicola* spp. 2 and 3 are probably *P. mystacalis* or a new species closely related to *P. mystacalis*. Another possibility is that these populations could be distinct species that have not accumulated detectable structural chromosomal polymorphisms and retain high karyotypic similarity. Analyses of additional characteristics certainly will improve the systematics of these populations and help to distinguish them from *P. mystacalis*.

The morphology of pair 7 and the difference in the distribution patterns of the heterochromatic C-bands distinguished *P. ternetzi* from *P. ameghini* (sensu Cope 1887). Therefore, the cytogenetic results strongly support the hypothesis that they belong to distinct taxonomic units. That contradicts the synonymization of *P. ternetzi* and *P. ameghini* proposed by Bokermann (1966), but not accepted by Lynch (1971).

The previously proposed taxonomic relatedness of *Pseudopaludicola* with the other genera (*Physalaemus*, *Pleurodema*, or *Edalorhina*) of the family Leptodactylidae (sensu Laurent 1986) was inferred from morphological data (Lynch 1971, Heyer 1974 1975, Cannatella and Duellman

1984, Lobo 1996). The evidence; however, even that based on molecular characteristics (see Frost et al. 2006 and Grant et al. 2006), is not conclusive, because no particular genus was definitely indicated as being closely related to *Pseudopaludicola*. Considerable intra-generic variability in chromosome numbers of *Pseudopaludicola* was described in this paper and in previous studies (Saez and Brum 1960, Beçak 1968, Brum-Zorrilla and Saez 1968, Batistic et al. 1969 1970, Batistic 1970, Duarte et al. 2010). Nevertheless, it seems premature to suggest any chromosomal homology that could corroborate the close taxonomic proximity of the genus *Pseudopaludicola* with genera of the family Leiuperidae, as hypothesized by Grant et al. (2006), especially *Physalaemus* (2*n* = 22), *Pleurodema* (2*n* = 22), and *Edalorhina* (2*n* = 22) (Lourenço et al. 1998 1999 2000ab 2006), or with genera of the family Leptodactylidae (Frost et al. 2006), such as *Paratelmatobius* (2*n* = 24) and *Scythrophrys* (2*n* = 24) (Lourenço et al. 2003 a b c 2008).

The presence of 2*n* = 18 chromosomes only in *P. canga* and in closely related species (Duarte et al. 2010) corroborates the monophyletism of the *P. pusilla* group previously inferred from morphological (Lynch 1989) and molecular data (Veiga-Menoncello et al. 2007). Additional phylogenetic studies may complement the cytogenetic data and improve our understanding of the inter- and intrageneric relatedness, as well as the variability in the number of chromosomes in *Pseudopaludicola*.

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REFERENCES

Batistic RF. 1970. Estudo cromossômico e mecanismos de especiação em *Pseudopaludicola* (Leptodactylidae, Anura). São Paulo, Brazil: Instituto de Biociências da Univ. de São Paulo.

Batistic RF, ML Beçak, W Beçak, LD Vizotto. 1970. Especiação e variação cariotípica em *Pseudopaludicola* (Amphibia, Anura). Salvador, Bahia, Brazil: Resumos da XXII Reunião Anual da Sociedade Brasileira para o Progresso da Ciência, E-72, 117 pp.

Batistic RF, ML Beçak, LD Vizotto. 1969. Variação cromossômica no gênero *Pseudopaludicola* (Anura). Ciên. Cult. **21:** 260.

Beçak ML 1968. Chromosomal analysis of eighteen species of Anura. Caryologia **21:** 191-208.

Bokermann WCA. 1966. Lista anotada das localidades tipo de anfíbios brasileiros. São Paulo, Brazil: Impresso pelo Serviço de Documentação, RUSP.

Brum-Zorrila N, FA Saez. 1968. Chromosomes of Leptodactylidae (Amphibian, Anura). Experientia **24:** 969.

Cannatella DC, WE Duellman. 1984. Leptodactylid frogs of the genus *Physalaemus postulosus* group. Copeia **4:** 902-921.

Cope ED. 1887. Synopsis of the Batrachia and Reptilia obtained by H. H. Smith, in the province of Mato Grosso, Brazil. Proc. Am. Phil. Soc. **24:** 44-60.

Duarte TC, ACP Veiga-Menoncello, JR Lima, C Strüssmann, ML Del-Grande, AA Giaretta et al. 2010. Chromosome analysis in *Pseudopaludicola* (Anura, Leiuperidae), with description of sex chromosomes XX/XY in *P. saltica*. Hereditas **147:** 43-52.

Frost DR. 2011. Amphibian species of the world: an online reference. Vers. 5.5 New York, NY: American Museum of Natural History. Available at http://research.amnh.org/vz/herpetology/amphibia Accessed 31 Jan. 2011.

Frost DR, T Grant, J Faivovich, RH Bain, A Haas, CFB Haddad et al. 2006. The amphibian tree of life. B. Am. Mus. Nat. Hist. **297:** 1-370.

Grant T, DR Frost, JP Caldwell, R Gagliardo, CFB Haddad, PJR Kok et al. 2006. Phylogenetic systematics of dart poison frogs and their relatives (Amphibia, Athesphatanura, Dendrobatidae). B. Am. Mus. Nat. Hist. **299:** 1-262.

Green MD, SK Sessions. 1991. Nomenclature for chromosomes. *In* DM Green, SK Sessions, eds. Amphibiam cytogenetics and evolution. San Diego, CA: Academic Press, pp. 431-432.

Haddad CFB, AJ Cardoso. 1987. Taxonomia de três espécies de *Pseudopaludicola* (Anura, Leptodactylidae). Pap. Avul. Zool. **36:** 287-300.

Heyer WR. 1974. Relationships of the *marmoratus* species group (Amphibia, Leptodactylidae) within the subfamily Leptodactylinae. Contr. Sci. Nat. Hist. Mus. **253:** 1-46.

Heyer WR. 1975. A preliminary analysis of the intergeneric relationships of the frog family Leptodactylidae. Smithson. Contr. Zool. **199:** 1-55.

Howell WM, DA Black. 1980. Controlled silver staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia **36:** 1014-1015.

King M, R Rofe. 1976. Karyotypic variation in the Australian gecko *Phyllodactylus marmoratus* (Gray) (Gekkonidae: Reptilia). Chromosoma **54:** 75-87.

Laurent RF. 1986. Sous classe des lissamphibiens. Sytématique. *In* PP Grassé, M Delsol, eds. Traité de Zoologie. Paris: Masson, pp. 459-797.

Lobo F. 1992. Distribución y lista de localidades de *Pseudopaludicola* (Anura: Leptodactylidae) en la República Argentina. Cuad. Herp. **7:** 30-37.

Lobo F. 1996. Evaluación del status taxonómico de *Pseudopaludicola ternetzi* Miranda Ribeiro, 1937 y *Pseudopaludicola mystacalis* (Cope, 1887). Osteologia y distribución de las dos especies. Acta Zool. Lilloana **43:** 327-346.

Lourenço LB, JA Andrade, GV Andrade, DC Rossa-Feres, SM Recco-Pimentel. 2006. Chromosomal analysis of the leptodactylids *Pleurodema diplolistris* and *Physalaemus nattereri* (Amphibia, Anura). Amphibia-Reptilia **27:** 481-489.

Lourenço LB, M Bacci-Júnior, VG Martins, SM Recco-Pimentel, CFB Haddad. 2008. Molecular phylogeny and karyotypic differentiation in *Paratelmatobius* and *Scythrophrys* (Anura, Leptodactylidae). Genetica **132:** 255-266.

Lourenço LB, AJ Cardoso, SM Recco-Pimentel. 2000a. Cytogenetics of *Edalorhina perezi* (Anura, Leptodactylidae). Cytologia **65:** 359-363.

Lourenço LB, PC Garcia, SM Recco-Pimentel. 2000b. Cytogenetics of two species of *Paratelmatobius* (Anura, Leptodactylidae) with phylogenetic comments. Hereditas **113:** 201-209.

Lourenço LB, PC Garcia, SM Recco-Pimentel. 2003a. Intrageneric karyotypic divergence in *Scythrophrys* (Anura, Leptodactylidae) and new insights on the relationship with the leptodactylid *Paratelmatobius*. Ital. J. Zool. **70:** 183-190.

Lourenço LB, PC Garcia, SM Recco-Pimentel. 2003b. Cytogenetics of a new species of the *Paratelmatobius cardosoi* group (Anura: Leptodactylidae) with the description of an apparent case of pericentric inversion. Amphibia-Reptilia **24:** 47-55.

Lourenço LB, PC Garcia, SM Recco-Pimentel. 2003c. Restriction fragment analysis of the ribosomal DNA of *Paratelmatobius* and *Scythrophrys* species (Anura: Leptodactylidae). Genet. Mol. Biol. **26:** 139-143.

Lourenço LB, SM Recco-Pimentel, AJ Cardoso. 1998. Polymorphism of the nucleolar organizer regions (NORs) in *Physalaemus petersi* (Amphibia, Anura, Leptodactylidae) detected by silver staining and fluorescence *in situ* hybridization. Chromosome Res. **6:** 621-628.

Lourenço LB, SM Recco-Pimentel, AJ Cardoso. 1999. Two karyotypes and heteromorphic sex chromosomes in *Physalaemus petersi* (Leptodactylidae, Anura). Can. J. Zool. **77:** 624-631.

Lynch JD. 1989. A review of the leptodactylid frogs of the genus *Pseudopaludicola* in northern South America. Copeia **3:** 577-588.

Lynch JD. 1971. Evolutionary relationships, osteology and zoogeography of leptodactyloid frogs. M. Nat. Hist. Univ. Kansas Misc. Publ. **53:** 1-238.

Medeiros LR, DC Rossa-Feres, SM Recco-Pimentel. 2003. Chromosomal differentiation of *Hyla nana* and *Hyla sanborni* (Anura, Hylidae) with a description of NOR polymorphism in *H. nana*. J. Hered. **94:** 149-154.

Milstead WW. 1963. Notes on Brazilian frogs of the genera *Physalaemus* and *Pseudopaludicola*. Copeia **1963:** 565-566.

Miranda-Ribeiro A. 1926. Notas para servirem ao estudo dos Gymnobatrachios (Anura) brasileiros. Arch. Mus. Nac. Rio de Janeiro Tomo I **27:** 1-227.

Saez FA, N Brum. 1960. Chromosomes of South American amphibians. Nature **185:** 945.

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

Siqueira S, O Aguiar-Jr, C Strüssmann, ML Del-Grande, SM Recco-Pimentel. 2008. Chromosomal analysis of three Brazilian "eleutherodactyline" frogs (Anura: Terrarana), with suggestion of a new species. Zootaxa **1860:** 51-59.

Strüssmann C. 2003. Herpetofauna da área sob influência do reservatório de Manso (Chapada dos Guimarães, Mato Grosso, Brasil): composição taxonômica, padrões de abundância e de distribuição em diferentes unidades de paisagem. PhD dissertation, Pontifícia Univ. Católica do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil.

Sumner AT. 1972. A simple technique for demonstrating centromeric heterochromatin. Exp. Cell. Res. **83:** 438-442.

Veiga-Menoncello ACP, C Strüssmann, AP Lima, SM Recco-Pimentel. 2006. Chromosomal study of *Colostethus brunneus* from the type locality and two related species (Anura, Dendrobatidae). Genetica **126:** 179-187.

Veiga-Menoncello ACP, ER Fávero, TC Duarte, LB Lourenço, C Stüssmann, DC Rossa-Feres et al. 2007. A progressive reduction of chromosome number in *Pseudopaludicola* (Anura, Leiuperidae), inferred from the molecular phylogenetic analysis. Chromosome Res. **15(Supplement):** 45-46.