Karyotypic Data on 28 Species of *Scinax* (Amphibia: Anura: Hylidae): Diversity and Informative Variation

Author(s) :Dario E. Cardozo, Daniela M. Leme, João F. Bortoleto, Glaucilene F. Catroli, Diego Baldo, Julián Faivovich, Francisco Kolenc, Ana P. Z. Silva, Claudio Borteiro, Célio F. B. Haddad, and Sanae Kasahara

Source: Copeia, 2011(2):251-263. 2011.
Published By: The American Society of Ichthyologists and Herpetologists
DOI:
URL: http://www.bioone.org/doi/full/10.1643/CH-09-105

Karyotypic Data on 28 Species of *Scinax* (Amphibia: Anura: Hylidae): Diversity and Informative Variation

Dario E. Cardozo[1], Daniela M. Leme[2], João F. Bortoleto[2], Glaucilene F. Catroli[2], Diego Baldo[3,4], Julián Faivovich[5], Francisco Kolenc[6], Ana P. Z. Silva[7], Claudio Borteiro[6], Célio F. B. Haddad[8], and Sanae Kasahara[2]

The hylid frog genus *Scinax* is the most species-rich within Hylinae, with more than 100 recognized species grouped in the *S. catharinae* and *S. ruber* clades. The karyotypes of 14 species of the *S. catharinae* clade and 14 of the *S. ruber* clade were analyzed, several of them for the first time. All studied species presented 2n = 2x = 24 biarmed chromosomes (FN = 48) and no identifiable sex chromosomes. There are two alternate states associated with the size and morphology of pair 1, corresponding to the *S. catharinae* clade and to the *S. ruber* clade. The morphology of pairs 2 and 6 also differentiate the species of both major clades. Species of the *S. ruber* clade in general have Ag-NORs in pair 11, as is commonly observed among hylines with 2n = 24. The Ag-NORs' position in the long arms of pair 11 is interstitial in *S. fuscomarginatus, S. fuscovarius, S. nasicus, S. similis, S. squalirostris*, and *S. uruguayus*, and terminal in *S. acuminatus, S. curicica, S. duartei, S. granulatus, S. hayii*, and *S. perereca*. The single exception among species of the *S. ruber* clade is *S. alter*, which has terminal Ag-NORs at the long arms of pair 3. Most species of the *S. catharinae* clade have Ag-NORs in pair 6, representing a putative synapomorphy of this clade, while the Ag-NORs in pair 11 that occur in *S. canastrensis* are most parsimoniously interpreted as a reversion. C-banding is predominantly centromeric, but in the *S. catharinae* clade there is a greater amount of heterochromatin than in the *S. ruber* clade. This study corroborates the occurrence of informative variation, some already considered in a previous cladistic analysis, and reports new characters, outlining the significance of cytogenetic data for the systematics of *Scinax*.

THE hylid frog genus *Scinax* comprises more than 100 species (Frost, 2011), distributed from southern Mexico to east-central Argentina, with the largest number of species occurring in the Atlantic forest of southeastern Brazil (Pombal et al., 1995; Faivovich, 2002; Frost, 2011). This genus has been the subject of several revisions (see taxonomic history in Faivovich, 2002). The recognition of two major clades (*S. catharinae* and *S. ruber* clades) has been supported by analyses based on adult and larval morphology, osteology, myology, reproductive biology, chromosome morphology, and molecular data (Faivovich, 2002; Faivovich et al., 2005). The *S. catharinae* clade includes the *S. catharinae* and the *S. perpusillus* groups and the *S. ruber* clade includes the *S. rostratus* and the *S. uruguayus* groups, as well as more than 40 species which remain unassigned to any group of the *S. ruber* clade.

Chromosome information is available for 19 species of *Scinax*, mostly derived from standard staining methods (Duellman, 1967; Rabello et al., 1971; Nunes and Fagundes, 2008). Although the studied species of *Scinax* have very similar 2n = 2x = 24 karyotypes, some conspicuous differences have been observed, mainly in regard to the morphology of pair 1 and the location of Ag-NORs. The goal of the present study is to improve our knowledge on the cytogenetics of *Scinax*, provide new information that allows re-evaluation of available data, and enlarge the data set of chromosome characters that would be useful for the study of chromosome evolution in this genus. The standard stained karyotypes of 28 species of *Scinax* are described, 20 of them for the first time. In addition, differential staining (C-banding and Ag-NORs) is used for many of these species. The results are discussed in the light of our current phylogenetic knowledge of the genus (Faivovich, 2002; Faivovich et al., 2005).

MATERIALS AND METHODS

We karyotyped 170 specimens belonging to 28 species of *Scinax* of both recognized major clades (Table 1). Institutional abbreviations follow Leviton et al. (1985), with the addition of CFBH for Célio F. B. Haddad Collection, Rio Claro, São Paulo, Brazil, and ZVC-B for Vertebrate Zoology Collection, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay. Chromosome spreads were prepared directly from intestinal epithelium or bone marrow, liver, and testis (Schmid, 1978; Baldissera et al., 1993). Mitotic and meiotic chromosome preparations were stained with a Giemsa-PBS solution (pH 6.8). The silver-staining of nucleolar organizer regions (Ag-NORs) and C-banding were performed according to Howell and Black (1980) and Sumner (1972), respectively.

[1] CONICET–IBIGEO, Museo de Ciencias Naturales, Universidad Nacional de Salta, Mendoza 2, 4400, Salta, Argentina; E-mail: darcardz@gmail.com.
[2] Departamento de Biologia, Instituto de Biociências, Universidade Estadual Paulista, UNESP, Av. 24A, 1515, 13506 900, Rio Claro, SP, Brasil; E-mail: (SK) kasahara@rc.unesp.br; and (GFC) gcatroli@gmail.com.
[3] Laboratorio de Genética Evolutiva, Facultad de Ciencias Exactas, Químicas y Naturales, Universidad Nacional de Misiones, Félix de Azara 1552, 3300 Posadas, Misiones, Argentina; E-mail: diegobaldo@gmail.com. Send reprint requests to this address.
[4] Instituto de Herpetología, Fundación Miguel Lillo, Miguel Lillo 251, CP: 4000 San Miguel de Tucumán, Argentina.
[5] División Herpetología, Museo Argentino de Ciencias Naturales ''Bernardino Rivadavia''—CONICET, Ángel Gallardo 470, 1405 Buenos Aires, Argentina; E-mail: julian@macn.gov.ar.
[6] Sección Herpetología, Museo Nacional de Historia Natural, 25 de Mayo 582, Montevideo, Uruguay; E-mail: (FK) fkolenc@gmail.com; and (CB) borteiro@gmail.com.
[7] Laboratório de Ecologia e Evolução, Instituto Butantan, Av. Vital Brasil 1500, 05503 900, São Paulo, SP, Brasil; E-mail: pietri@uol.com.br.
[8] Departamento de Zoologia, Instituto de Biociências, Universidade Estadual Paulista, UNESP, Av. 24A, 1515, 13506 900, Rio Claro, SP, Brasil; E-mail: haddad1000@gmail.com.
Submitted: 8 June 2009. Accepted: 6 January 2011. Associate Editor: T. W. Reeder.
 DOI: 10.1643/CH-09-105

Table 1. Morphometric Analysis of the Chromosomes of Species of *Scinax* of the Present Study, Including Clade and Species Group. RL = Relative length, CR = Centromeric ratio, CI = Centromeric index, CT = Chromosome type: *m* = metacentric, *sm* = submetacentric, and *st* = subtelocentric.

Clade (Group)	Species		Chromosome pair											
			1	2	3	4	5	6	7	8	9	10	11	12
S. catharinae (*S. catharinae*)	*S. albicans* (Bokermann, 1967)	RL	12.12	10.98	10.80	10.24	9.71	8.73	8.62	7.02	6.22	5.48	5.26	4.82
		CR	2.89±0.07	2.43±0.08	3.15±0.02	4.43±0.71	4.30±0.05	3.67±0.21	3.39±0.11	1.62±0.42	1.41±0.43	1.36±0.36	1.13±0.15	1.09±0.08
		CI	0.26±0.01	0.29±0.01	0.24±0.01	0.19±0.02	0.19±0.02	0.21±0.01	0.23±0.01	0.39±0.06	0.42±0.08	0.43±0.06	0.47±0.03	0.48±0.02
		CT	*sm*	*sm*	*st*	*st*	*st*	*st*	*st*	*m*	*m*	*m*	*m*	*m*
	S. argyreornatus (Miranda-Ribeiro, 1926)	RL	11.07	10.9	10.9	10.49	9.88	9.01	7.35	6.74	6.25	6.19	6.18	5.04
		CR	2.01±0.30	2.02±0.53	2.88±0.27	1.88±0.26	1.81±0.08	3.36±0.08	2.55±0.37	1.34±0.16	1.04±0.04	1.02±0.01	1.16±0.17	1.04±0.10
		CI	0.33±0.03	0.34±0.06	0.26±0.02	0.35±0.03	0.36±0.01	0.23±0.01	0.28±0.03	0.43±0.03	0.49±0.01	0.49±0.01	0.46±0.04	0.49±0.02
		CT	*sm*	*sm*	*sm*	*sm*	*sm*	*st*	*sm*	*m*	*m*	*m*	*m*	*m*
	S. ariadne (Bokermann, 1967)	RL	13.67	11.58	11.03	10.61	9.39	8.66	7.55	5.83	5.78	5.70	5.54	4.66
		CR	2.63±0.13	2.36±0.15	2.43±0.27	1.91±0.17	2.50±0.39	3.07±0.18	2.66±0.27	1.03±0.04	1.07±0.02	1.26±0.14	1.14±0.15	1.07±0.04
		CI	0.28±0.01	0.30±0.01	0.28±0.02	0.34±0.02	0.29±0.03	0.25±0.01	0.28±0.02	0.49±0.02	0.49±0.01	0.44±0.03	0.49±0.01	0.48±0.01
		CT	*sm*	*sm*	*sm*	*sm*	*sm*	*st*	*sm*	*m*	*m*	*m*	*m*	*m*
	S. aromothyella Faivovich, 2005	RL	11.07	10.9	10.9	10.49	9.88	9.01	7.35	6.74	6.25	6.19	6.18	5.04
		CR	2.18±0.23	2.04±0.23	2.50±0.12	2.76±0.25	2.48±0.25	3.17±0.08	2.10±0.10	1.32±0.16	1.17±0.14	1.37±0.11	1.11±0.08	1.37±0.10
		CI	0.32±0.02	0.33±0.03	0.29±0.01	0.27±0.03	0.28±0.02	0.24±0.01	0.32±0.02	0.43±0.03	0.46±0.03	0.42±0.02	0.47±0.02	0.42±0.02
		CT	*sm*	*sm*	*sm*	*sm*	*sm*	*st*	*sm*	*m*	*m*	*m*	*m*	*m*
	S. berthae (Barrio, 1962)	RL	12.63	11.52	10.50	9.12	9.81	8.33	8.13	6.73	6.40	6.11	5.53	5.19
		CR	2.50±0.11	2.55±0.07	2.32±0.19	2.81±0.31	2.43±0.15	3.40±0.25	2.72±0.22	1.33±0.16	1.12±0.11	1.32±0.23	1.21±0.12	1.31±0.10
		CI	0.29±0.01	0.28±0.01	0.30±0.02	0.26±0.02	0.29±0.01	0.23±0.01	0.27±0.02	0.43±0.03	0.47±0.02	0.43±0.04	0.45±0.02	0.43±0.02
		CT	*sm*	*sm*	*sm*	*sm*	*sm*	*st*	*sm*	*m*	*m*	*m*	*m*	*m*
	S. canastrensis (Cardoso and Haddad, 1982)	RL	12.18	10.65	10.61	10.54	9.45	8.53	7.59	7.42	7.04	5.84	5.72	4.43
		CR	2.19±0.17	2.35±0.10	1.76±0.24	2.83±0.07	1.14±0.11	2.34±0.23	2.09±0.20	1.35±0.14	1.61±0.08	1.19±0.22	1.06±0.07	1.31±0.15
		CI	0.31±0.02	0.30±0.01	0.36±0.03	0.26±0.01	0.11±0.02	0.27±0.04	0.32±0.02	0.43±0.03	0.37±0.01	0.46±0.05	0.46±0.06	0.43±0.03
		CT	*sm*	*sm*	*sm*	*sm*	*m*	*sm*	*sm*	*m*	*m*	*m*	*m*	*m*
	S. hiemalis (Haddad and Pombal, 1987)	RL	12.85	11.41	11.05	10.71	10.11	9.69	8.12	6.06	5.67	5.53	4.53	4.27
		CR	2.62±0.48	2.44±0.26	2.86±0.37	2.15±0.16	2.67±0.42	3.44±0.20	2.79±0.09	1.30±0.35	1.16±0.17	1.62±0.21	1.25±0.04	1.10±0.07
		CI	0.28±0.04	0.28±0.04	0.31±0.06	0.30±0.03	0.28±0.04	0.23±0.01	0.26±0.01	0.44±0.06	0.46±0.03	0.38±0.03	0.44±0.01	0.48±0.02
		CT	*sm*	*sm*	*sm*	*sm*	*sm*	*st*	*sm*	*m*	*m*	*m*	*m*	*m*
	S. littoralis (Pombal and Gordo, 1991)	RL	11.77	10.77	10.48	9.91	9.48	8.63	8.52	7.21	6.65	6.14	5.55	4.89
		CR	2.62±0.16	2.01±0.18	2.80±0.18	2.86±0.21	2.79±0.19	3.35±0.36	2.49±0.30	1.96±0.26	1.55±0.39	1.18±0.10	1.15±0.09	1.15±0.06
		CI	0.28±0.01	0.33±0.02	0.26±0.01	0.26±0.01	0.26±0.01	0.23±0.02	0.29±0.02	0.34±0.03	0.40±0.06	0.46±0.02	0.47±0.02	0.47±0.01
		CT	*sm*	*sm*	*sm*	*sm*	*sm*	*st*	*sm*	*m*	*m*	*m*	*m*	*m*
	S. longilineus (Lutz, 1968)	RL	11.69	10.94	10.64	9.66	9.44	8.48	8.34	7.13	6.37	6.19	5.66	5.46
		CR	2.59±0.32	2.37±0.37	2.55±0.33	2.45±0.27	2.14±0.24	3.13±0.48	2.52±0.28	1.53±0.19	1.29±0.16	1.31±0.28	1.14±0.13	1.18±0.11
		CI	0.28±0.02	0.30±0.03	0.28±0.03	0.29±0.02	0.32±0.02	0.25±0.02	0.29±0.02	0.40±0.03	0.44±0.03	0.44±0.05	0.47±0.03	0.46±0.02
		CT	*sm*	*sm*	*sm*	*sm*	*sm*	*st*	*sm*	*m*	*m*	*m*	*m*	*m*
	S. obtriangulatus (Lutz, 1973)	RL	12.71	10.65	10.20	9.90	9.54	8.31	7.99	7.30	6.73	6.04	5.32	5.31
		CR	2.25±0.03	2.86±0.22	2.29±0.09	2.78±0.32	2.64±0.14	3.15±0.03	2.39±0.18	1.17±0.14	1.39±0.15	1.25±0.27	1.13±0.01	1.07±0.04
		CI	0.31±0.01	0.26±0.02	0.30±0.01	0.27±0.02	0.28±0.01	0.24±0.01	0.24±0.01	0.46±0.03	0.42±0.03	0.45±0.05	0.47±0.01	0.48±0.01
		CT	*sm*	*sm*	*sm*	*sm*	*sm*	*st*	*sm*	*m*	*m*	*m*	*m*	*m*

Table 1. Continued.

Clade (Group)	Species		Chromosome pair											
			1	2	3	4	5	6	7	8	9	10	11	12
	S. rizibilis (Bokermann, 1964)	RL	12.24	11.83	10.67	10.24	9.57	8.38	7.78	6.89	6.30	6.06	5.62	4.42
		CR	2.76±0.20	2.26±0.17	2.70±0.24	2.55±0.36	2.61±0.25	3.43±0.17	2.83±0.22	1.38±0.28	1.26±0.24	1.12±0.09	1.14±0.02	1.12±0.10
		CI	0.27±0.01	0.31±0.02	0.27±0.02	0.28±0.03	0.28±0.02	0.23±0.01	0.26±0.02	0.42±0.05	0.45±0.05	0.47±0.02	0.47±0.02	0.47±0.02
		CT	*sm*	*sm*	*sm*	*sm*	*sm*	*st*	*sm*	*m*	*m*	*m*	*m*	*m*
	S. trapicheiroi (A. Lutz and B. Lutz *in* Lutz, 1954)	RL	12.36	12.13	11.76	9.69	9.52	8.70	8.06	6.18	5.96	5.94	5.21	4.49
		CR	2.59±0.03	2.64±0.31	2.47±0.66	2.94±0.52	2.61±0.25	3.21±0.36	2.57±0.22	1.18±0.28	1.23±0.24	1.35±0.09	1.09±0.02	1.12±0.10
		CI	0.28±0.03	0.23±0.04	0.28±0.05	0.26±0.02	0.28±0.02	0.24±0.02	0.28±0.02	0.42±0.05	0.45±0.05	0.47±0.02	0.47±0.02	0.47±0.02
		CT	*sm*	*sm*	*sm*	*sm*	*sm*	*st*	*sm*	*m*	*m*	*m*	*m*	*m*
S. catharinae (*S. perpusillus*)	*Scinax* sp. 1	RL	12.38	12.01	11.07	10.50	9.56	9.27	8.45	6.54	6.08	5.31	4.64	4.19
		CR	2.05±0.16	2.33±0.27	3.51±0.13	4.43±0.03	3.84±0.24	4.74±0.10	3.45±0.02	1.69±0.23	1.40±0.18	1.20±0.22	1.47±0.02	1.15±0.09
		CI	0.33±0.02	0.25±0.05	0.27±0.07	0.18±0.01	0.21±0.01	0.21±0.01	0.22±0.01	0.34±0.03	0.34±0.03	0.46±0.04	0.40±0.01	0.47±0.02
		CT	*sm*	*sm*	*st*	*st*	*st*	*st*	*st*	*m*	*m*	*m*	*m*	*m*
	Scinax sp. 2	RL	12.45	11.95	10.98	10.69	9.63	9.30	8.38	6.32	6.21	5.28	4.68	4.13
		CR	2.62±0.48	2.44±0.26	2.86±0.37	2.15±0.16	2.67±0.42	3.44±0.20	2.79±0.09	1.30±0.35	1.16±0.17	1.62±0.21	1.25±0.04	1.10±0.07
		CI	0.28±0.04	0.28±0.04	0.31±0.06	0.30±0.03	0.28±0.04	0.23±0.01	0.26±0.01	0.44±0.06	0.46±0.03	0.38±0.03	0.44±0.01	0.48±0.02
		CT	*sm*	*sm*	*sm*	*sm*	*sm*	*st*	*sm*	*m*	*m*	*m*	*m*	*m*
S. ruber (unassigned to any group)	*S. acuminatus* (Cope, 1862)	RL	16.57	11.83	10.37	9.77	8.75	8.38	6.61	6.34	5.80	5.62	5.16	4.80
		CR	1.19±0.11	1.56±0.05	2.04±0.20	2.55±0.27	2.44±0.26	2.42±0.17	2.02±0.08	1.15±0.11	1.25±0.09	1.26±0.14	1.30±0.18	1.09±0.09
		CI	0.46±0.02	0.39±0.01	0.33±0.02	0.28±0.02	0.29±0.02	0.29±0.01	0.33±0.01	0.47±0.02	0.45±0.02	0.44±0.03	0.44±0.04	0.48±0.02
		CT	*m*	*m*	*sm*	*sm*	*sm*	*Sm*	*sm*	*m*	*m*	*m*	*m*	*m*
	S. alter (Lutz, 1973)	RL	14.7	12.86	10.78	10.49	8.64	7.84	7.00	6.26	6.13	5.92	4.84	4.54
		CR	1.27±0.11	1.21±0.29	2.03±0.67	2.35±0.37	2.23±0.15	2.29±0.44	1.33±0.08	1.29±0.15	1.09±0.05	1.07±0.08	1.48±0.28	1.14±0.08
		CI	0.44±0.02	0.46±0.06	0.34±0.07	0.30±0.03	0.31±0.01	0.31±0.04	0.43±0.01	0.44±0.03	0.48±0.01	0.48±0.02	0.41±0.05	0.47±0.02
		CT	*m*	*m*	*sm*	*sm*	*sm*	*sm*	*m*	*m*	*m*	*m*	*m*	*m*
	S. curicica Pugliese, Pombal, and Sazima, 2004	RL	16.08	11.54	10.35	10.63	9.33	8.25	6.55	6.21	5.82	5.55	5.46	4.23
		CR	1.10±0.09	1.48±0.14	1.90±0.41	2.55±0.28	2.45±0.32	2.41±0.37	1.76±0.41	1.30±0.29	1.17±0.16	1.17±0.11	1.18±0.18	1.18±0.18
		CI	0.48±0.02	0.40±0.02	0.35±0.05	0.28±0.02	0.29±0.03	0.30±0.03	0.37±0.02	0.44±0.05	0.45±0.03	0.46±0.02	0.46±0.03	0.46±0.03
		CT	*m*	*m*	*sm*	*sm*	*sm*	*sm*	*sm*	*m*	*m*	*m*	*m*	*m*
	S. duartei (Lutz, 1951)	RL	14.64	12.15	10.22	10.46	9.21	8.52	6.88	6.50	5.80	5.73	4.48	5.41
		CR	1.10±0.09	1.43±0.31	1.94±0.24	2.13±0.30	2.07±0.32	2.23±0.32	1.40±0.24	1.38±0.20	1.26±0.17	1.19±0.18	1.22±0.15	1.29±0.11
		CI	0.48±0.02	0.42±0.04	0.34±0.03	0.32±0.03	0.33±0.03	0.31±0.03	0.42±0.04	0.42±0.03	0.44±0.03	0.46±0.04	0.45±0.03	0.44±0.02
		CT	*m*	*m*	*sm*	*sm*	*sm*	*sm*	*m*	*m*	*m*	*m*	*m*	*m*
	S. eurydice (Bokermann, 1968)	RL	15.48	12.69	10.73	10.25	9.23	7.71	6.62	5.99	5.78	5.70	5.25	4.57
		CR	1.17±0.08	1.55±0.19	2.33±0.46	2.68±0.43	2.21±0.40	2.45±0.45	1.40±0.27	1.52±0.25	1.33±0.13	1.36±0.25	1.18±0.14	1.47±0.30
		CI	0.46±0.02	0.39±0.03	0.30±0.04	0.27±0.03	0.32±0.04	0.29±0.04	0.42±0.05	0.40±0.04	0.43±0.02	0.43±0.05	0.45±0.03	0.39±0.06
		CT	*m*	*m*	*sm*	*sm*	*sm*	*sm*	*m*	*m*	*m*	*m*	*m*	*m*
	S. fusco-marginatus (Lutz, 1925)	RL	15.35	11.66	10.00	9.30	8.54	8.01	7.38	7.20	6.39	6.32	5.67	4.18
		CR	1.10±0.08	1.31±0.07	1.81±0.14	2.30±0.18	2.24±0.28	2.39±0.35	1.86±0.19	1.12±0.06	1.11±0.11	1.25±0.07	1.97±0.20	1.11±0.08
		CI	0.48±0.02	0.43±0.01	0.36±0.02	0.30±0.02	0.31±0.02	0.30±0.03	0.35±0.02	0.47±0.01	0.48±0.02	0.45±0.01	0.34±0.02	0.47±0.02
		CT	*m*	*m*	*sm*	*sm*	*sm*	*sm*	*sm*	*m*	*m*	*m*	*sm*	*m*

Table 1. Continued.

Clade (Group)	Species		Chromosome pair											
			1	2	3	4	5	6	7	8	9	10	11	12
	S. fuscovarius (Lutz, 1925)	RL	14.68	11.02	10.88	10.76	9.2	8.31	6.61	6.22	6.22	5.99	5.62	4.49
		CR	1.15±0.09	1.34±0.19	2.06±0.11	2.85±0.09	2.40±0.19	2.73±0.26	2.15±0.16	1.29±0.23	1.68±0.10	1.15±0.11	1.98±0.25	1.85±0.23
		CI	0.47±0.02	0.43±0.04	0.33±0.01	0.24±0.01	0.30±0.02	0.23±0.01	0.32±0.02	0.44±0.04	0.36±0.01	0.46±0.02	0.34±0.03	0.35±0.03
		CT	*m*	*m*	*sm*	*sm*	*sm*	*sm*	*sm*	*m*	*m*	*m*	*sm*	*sm*
	S. granulatus (Peters, 1871)	RL	15.09	12.14	10.71	10.08	8.40	8.08	6.31	6.17	6.13	5.95	5.87	5.08
		CR	1.19±0.09	1.47±0.09	2.10±0.19	2.30±0.07	2.03±0.18	2.05±0.17	1.65±0.11	1.15±0.12	1.15±0.11	1.26±0.09	2.03±0.12	1.29±0.07
		CI	0.46±0.02	0.41±0.01	0.32±0.02	0.30±0.01	0.33±0.02	0.33±0.02	0.38±0.02	0.47±0.03	0.47±0.02	0.44±0.02	0.33±0.01	0.44±0.01
		CT	*m*	*m*	*sm*	*sm*	*sm*	*sm*	*m*	*m*	*m*	*m*	*sm*	*m*
	S. hayii (Barbour, 1909)	RL	16.07	13.06	10.37	10.23	8.90	8.17	6.70	6.21	5.56	5.19	4.49	5.05
		CR	1.07±0.05	1.42±0.09	2.82±0.14	2.29±0.27	2.12±0.34	2.17±0.08	1.80±0.14	1.24±0.09	1.18±0.08	1.23±0.19	1.13±0.12	1.26±0.22
		CI	0.48±0.01	0.41±0.02	0.26±0.01	0.31±0.02	0.32±0.04	0.32±0.01	0.36±0.02	0.45±0.02	0.46±0.02	0.45±0.04	0.47±0.03	0.45±0.04
		CT	*m*	*m*	*sm*	*sm*	*sm*	*sm*	*sm*	*m*	*m*	*m*	*m*	*m*
	S. nasicus (Cope, 1862)	RL	14.44	12.49	9.94	8.86	8.72	7.66	6.65	6.60	6.60	6.53	6.06	5.45
		CR	1.10±0.07	1.37±0.15	2.07±0.16	2.54±0.15	2.44±0.19	2.50±0.09	1.90±0.20	1.19±0.21	1.31±0.23	1.13±0.06	1.73±0.17	1.37±0.09
		CI	0.48±0.02	0.42±0.03	0.33±0.02	0.28±0.01	0.29±0.02	0.29±0.01	0.35±0.03	0.46±0.02	0.44±0.04	0.47±0.01	0.37±0.02	0.42±0.02
		CT	*m*	*m*	*sm*	*sm*	*sm*	*sm*	*sm*	*m*	*m*	*m*	*sm*	*m*
	S. perereca Pombal, Haddad, and Kasahara, 1995	RL	15.84	12.20	11.35	9.56	9.48	8.21	6.00	5.99	5.98	5.92	3.92	5.53
		CR	1.07±0.04	1.54±0.11	2.26±0.22	2.41±0.15	2.08±0.08	2.46±0.29	2.06±0.14	1.11±0.12	1.24±0.09	1.22±0.08	1.89±0.05	1.13±0.11
		CI	0.48±0.01	0.39±0.02	0.31±0.02	0.29±0.01	0.33±0.01	0.29±0.02	0.33±0.01	0.48±0.03	0.45±0.02	0.45±0.02	0.35±0.01	0.47±0.02
		CT	*m*	*m*	*sm*	*sm*	*sm*	*sm*	*sm*	*m*	*m*	*m*	*sm*	*m*
	S. similis (Cochran, 1952)	RL	15.76	13.34	11.21	10.40	8.10	7.81	6.69	6.09	5.44	5.25	5.19	4.72
		CR	1.11±0.08	1.38±0.09	2.00±0.18	2.68±0.07	2.31±0.37	2.28±0.16	1.83±0.06	1.23±0.16	1.20±0.15	1.25±0.15	2.00±0.12	1.19±0.12
		CI	0.47±0.02	0.42±0.02	0.33±0.02	0.27±0.01	0.31±0.01	0.31±0.02	0.35±0.01	0.45±0.03	0.46±0.03	0.45±0.03	0.33±0.01	0.46±0.03
		CT	*m*	*m*	*sm*	*sm*	*sm*	*sm*	*sm*	*m*	*m*	*m*	*sm*	*m*
	S. squalirostris (Lutz, 1925)	RL	14.94	12.49	9.32	9.31	8.98	8.41	6.80	6.69	6.68	5.59	5.65	5.13
		CR	1.14±0.09	1.47±0.16	2.01±0.10	2.50±0.04	2.24±0.01	2.38±0.14	1.89±0.09	1.17±0.08	1.21±0.08	1.25±0.02	2.04±0.08	1.28±0.07
		CI	0.47±0.02	0.41±0.03	0.33±0.01	0.29±0.01	0.31±0.01	0.30±0.01	0.35±0.01	0.46±0.02	0.45±0.02	0.44±0.01	0.33±0.01	0.44±0.01
		CT	*m*	*m*	*sm*	*sm*	*sm*	*sm*	*sm*	*m*	*m*	*m*	*sm*	*m*
S. ruber (*S. uruguayus*)	*S. uruguayus* (Schmidt, 1944)	RL	14.21	12.60	10.14	9.88	9.72	7.90	6.43	6.35	5.95	5.75	5.63	5.45
		CR	1.11±0.07	1.54±0.11	1.89±0.07	2.49±0.12	2.19±0.10	2.49±0.23	1.97±0.12	1.17±0.12	1.28±0.14	1.26±0.18	1.90±0.17	1.15±0.13
		CI	0.46±0.01	0.41±0.01	0.35±0.01	0.29±0.01	0.31±0.01	0.29±0.02	0.34±0.02	0.46±0.02	0.46±0.04	0.45±0.04	0.35±0.02	0.47±0.03
		CT	*m*	*m*	*sm*	*sm*	*sm*	*sm*	*sm*	*m*	*m*	*m*	*sm*	*m*

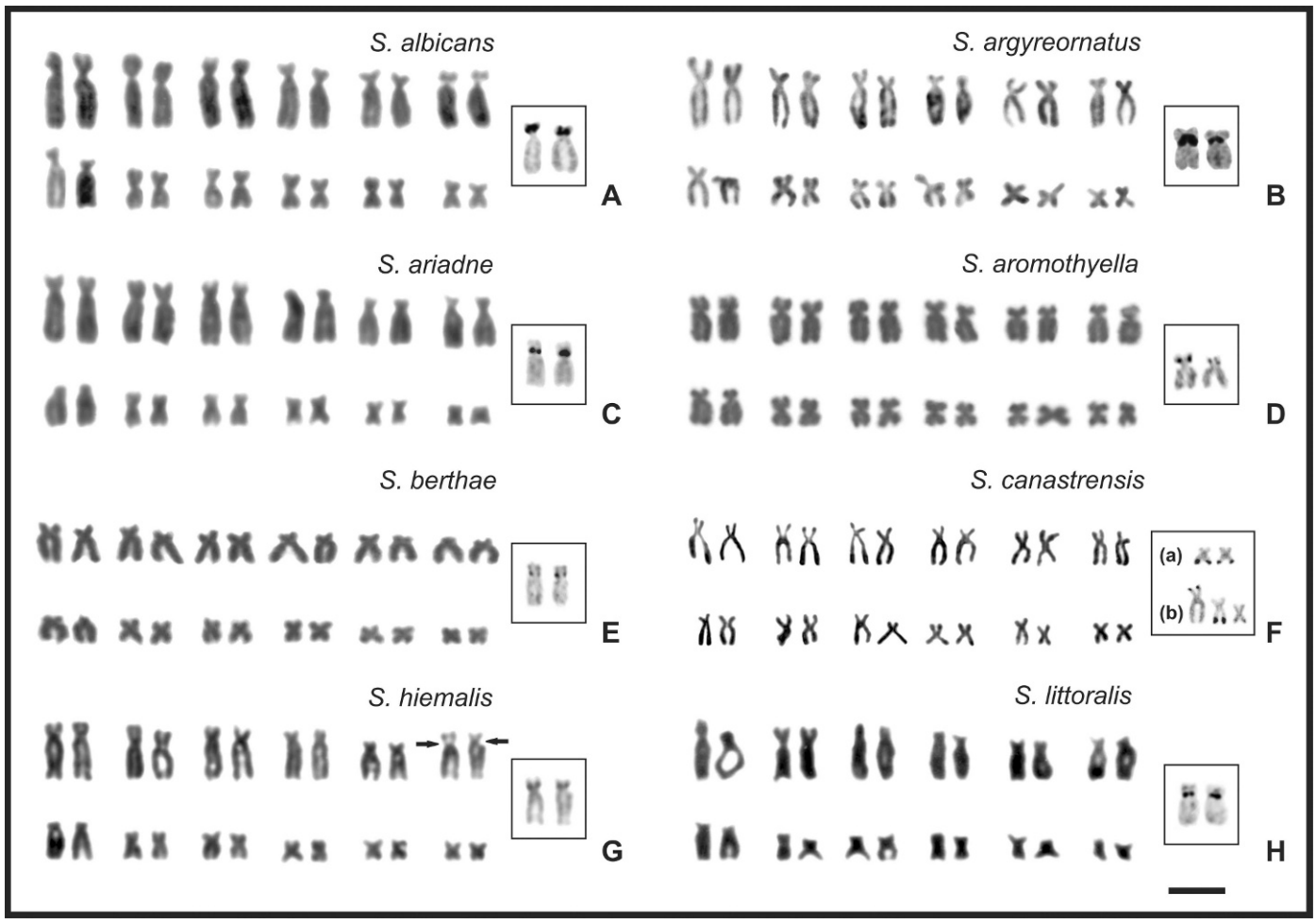

Fig. 1. Giemsa stained karyotypes and chromosomes after Ag-NOR technique (inset) of eight species of the *Scinax catharinae* clade assigned to the *S. catharinae* group. In A–E and G–H, Ag-NORs are in pair 6; in F(a,b), Ag-NORs are in pair 11, but in one female an additional Ag-labeling is seen at the *p* in one of the homologues of pair 6 (b). The arrows indicate the *sc*. Scale bar = 10 μm.

The morphometric measurements of chromosomes were made using Micromeasure v3.3 software (Reeves and Tear, 2000) and the mitotic chromosomes were arranged in decreasing size, following the nomenclature of Green and Sessions (1991, 2007) of metacentric (*m*), submetacentric (*sm*), or subtelocentric (*st*) for biarmed chromosomes. The relative length (RL), centromeric index (CI), and centromeric ratio (CR) were calculated. We used the term 'homoeology' to designate the homology present between chromosomes of different species that have descended from a common ancestral chromosome (Huskins, 1932). In this sense, although the chromosomes were arranged by decreasing size, this order was altered in the case of the third and fourth chromosome pairs of *S. curicica* and *S. duartei*; the second and third pairs of *S. albicans*; and the 11th and 12th pairs of *S. duartei*, *S. hayii,* and *S. perereca*, to indicate apparent homoeology. We used x (basic chromosome number), n (gametic chromosome number), 2n (somatic chromosome number), and FN (fundamental number of chromosome arms) as suggested by White (1954). Other abbreviations used are: NORs (nucleolar organizer regions); *p* (short arm); *q* (long arm); *sc* (secondary constrictions).

RESULTS

Description of karyotypes.—All 28 species of *Scinax* presented 2n = 24 chromosomes and FN of 48 chromosome arms, with six large or medium pairs, and six small-sized pairs (Figs. 1–4; Table 1). Although in 15 species we analyzed only one sex, cytologically heteromorphic sex chromosomes were not observed. The karyotypes of the 14 species of the *S. catharinae* clade were quite similar. The most divergent features were the *st* pairs 3, 4, 5, and 7 of *S. albicans* and *Scinax* sp. 1 (*perpusillus* group), which are *sm*-like in the remainder species of the clade, and only in *S. canastrensis* the fifth pair was *m*. Similarly, karyotypes of the 14 species of *S. ruber* clade were very similar, with some small differences in the chromosome pairs 7, 11, and 12. Karyotypes differed notably between these two major clades, with the first and second pairs being *m* in the *S. ruber* clade and *sm* in the *S. catharinae* clade, and the sixth pair being *sm* in the *S. ruber* clade and *st* in the *S. catharinae* clade. Intraspecific variation was minimal; only one specimen of *S. similis* (CFBH 5932) had heteromorphic pairs 3 and 4, probably due to a reciprocal translocation between them (Kasahara, pers. obs.). NOR-bearing chromosomes frequently presented *sc* in all studied species (Figs. 1G, 2C–E, 3D, 3G, 4A–D, 4F), very prominent in some (e.g., *S. rizibilis*, *Scinax* sp. 1 [*perpusillus* group], and *S. similis*; Figs. 2C, 2E, 4D). Males exhibited 12 bivalents in diplotene-diakinesis or metaphase I cells, most of them ring-shaped, with two terminal chiasmata. Metaphase II cells presented 12 chromosomes.

Differential staining.—Silver staining was performed in all species, except for *S. trapicheiroi*. In the *S. catharinae* clade,

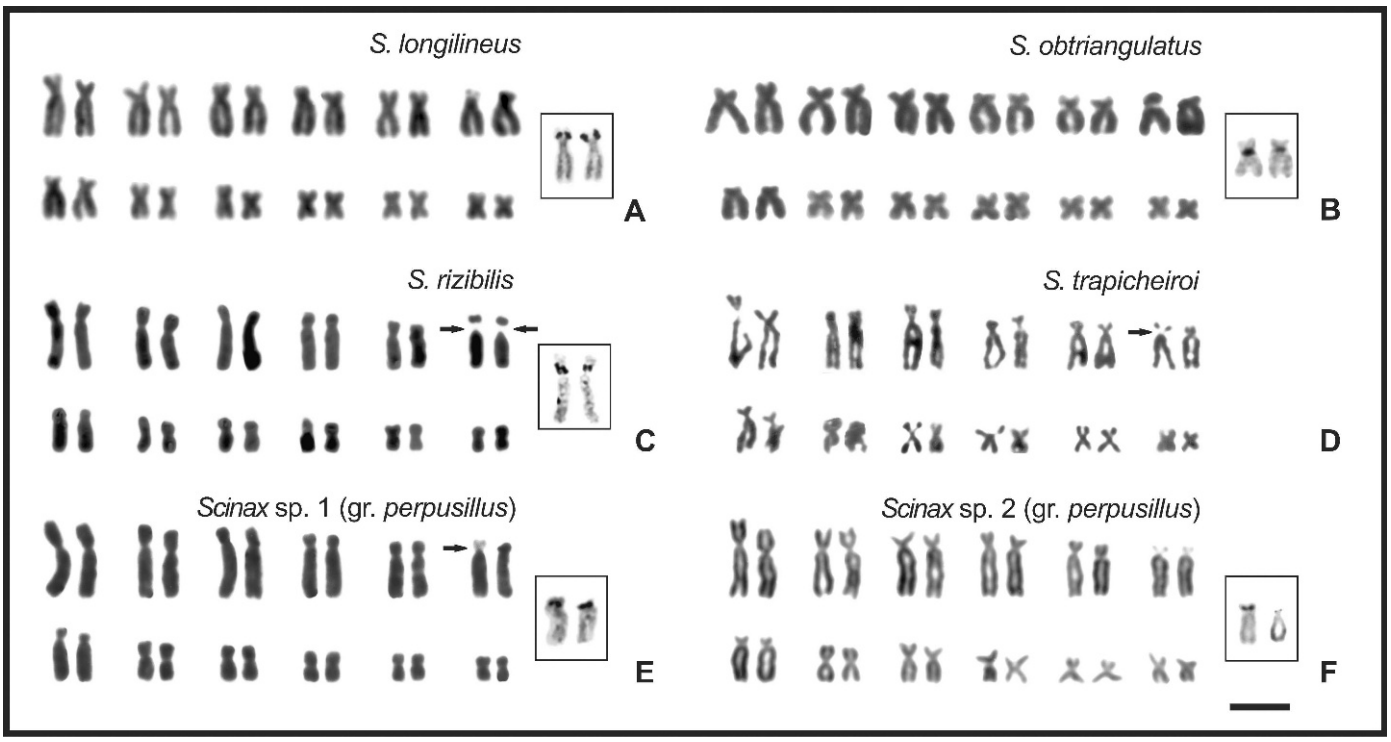

Fig. 2. Giemsa stained karyotypes and chromosomes after Ag-NOR technique (inset) of six species of the *Scinax catharinae* clade, four of them (A–D) assigned to the *S. catharinae* and two (E–F) to the *S. perpusillus* group. In A–C and E–F, Ag-NORs are in pair 6. The arrows indicate the *sc*. Scale bar = 10 μm.

Ag-NORs were located on pair 6*p*. They appeared interstitially in *S. albicans*, *S. aromothyella*, *S. hiemalis*, *S. longilineus*, *Scinax* sp. 1 (*perpusillus* group), and *Scinax* sp. 2 (*perpusillus* group), and proximally in *S. argyreornatus, S. ariadne*, *S. berthae*, *S. littoralis*, *S. obtriangulatus*, and *S. rizibilis* (Figs. 1, 2A–C, 2E–F). In two males of *S. canastrensis*, Ag-NORs were at the terminal of pair 11*q* (Fig. 1Fa), and a female (CFBH 16729) had an additional labeling at *p* of one of the homologues of pair 6, totaling three Ag-NORs in all analyzed metaphases (Fig. 1Fb). Most species of the *S. ruber* clade showed Ag-NORs on pair 11*q*. They were located at the terminal region in *S. acuminatus*, *S. curicica*, *S. duartei*, *S. granulatus*, *S. hayii*, and *S. perereca* (Figs. 3A, 3C–D, 3H, 4A, 4C), or in the proximal region in *S. fuscomarginatus*, *S. fuscovarius*, *S. nasicus*, *S. similis*, *S. squalirostris*, and *S. uruguayus* (Figs. 3F–G, 4B, 4D–F). In *S. eurydice*, Ag-NORs were terminal on pair 11*q* in three specimens (Fig. 3Eb), but in one female (CFBH 4003) the Ag+ labeling appeared in the proximal region (Fig. 3Ea). Another variation was observed in one male of *S. hayii* (unvouchered specimen) in which, in addition to the NORs-bearing pair 11, a terminal labeling was observed in *q* of one of the homologues of pair 8 (Fig. 4Ab). The single exception among studied species of the *S. ruber* clade is *S. alter*, which had terminal Ag-NORs on pair 3*q* (Fig. 3B). Heteromorphism related to size of Ag-NORs was frequently observed, and in one specimen of *S. alter* (CFBH 17293; Fig. 3Bb) and one of *S. hiemalis* (CFBH 16723), the labeling was reduced or almost indistinguishable and only a single Ag-NOR was seen per metaphase.

C-banding was obtained from most species, except for *S. fuscomarginatus*, *Scinax* sp. 1 (*perpusillus* group), *S. trapicheiroi*, and *S. uruguayus*. The banding pattern was predominantly centromeric (Figs. 5–8), with the amount of heterochromatin greater in some species of the *S. catharinae* group, such as *S. aromothyella*, *S. berthae*, *S. canastrensis*, and particularly in *S. longilineus* that showed very prominent pericentromeric C-positive blocks. Other species, mainly of the *S. ruber* clade, presented very subtle centromeric labeling of C-bands, which were missing in some chromosomes. Positive C-bands coincident with NORs sites were noticed in some karyotypes (e.g., *S. argyreornatus*, *S. curicica*, *S. eurydice*, *S. hiemalis*, *S. similis*, and *S. squalirostris*).

DISCUSSION

Differences in chromosome morphology between species of *Scinax* were initially pointed out by Bogart (1973), who analyzed *S. albicans* (as *S. catharinae*), *S. brieni*, *S. perpusillus*, *S. rostratus*, and *S. ruber*. He noticed that while karyotypes of *S. rostratus* and *S. ruber* were similar to most other species with 24 chromosomes then included in the genus *Hyla*, those of *S. albicans* and *S. brieni* had more *sm* chromosomes, and those of *S. perpusillus* could be considered intermediate between these two pairs of species. Faivovich (2002) compiled information on chromosome morphology of *Scinax*, and noticed that Bogart (1973) did not provide centromeric indexes, making it difficult to assess chromosome morphology from his plates, beyond the obvious case of pair 1. For this reason, only the *sm* or *m* morphology of pair 1 of *S. albicans*, *S. brieni, S. elaeochrous*, *S. fuscomarginatus*, *S. fuscovarius*, *S. hayii*, *S. perereca*, *S. perpusillus*, *S. rostratus*, *S. ruber*, *S. squalirostris*, and *S. staufferi* (Beçak, 1968; Barrio and Pistol de Rubel, 1970; Rabello, 1970; Anderson, 1991; Baldissera et al., 1993; Pombal et al., 1995) was included as a character by Faivovich (2002) in his phylogenetic analysis, which resulted in the recognition of the two major clades of *Scinax*.

The present study reports more informative variation regarding the chromosome morphology of *Scinax*. Pairs 1

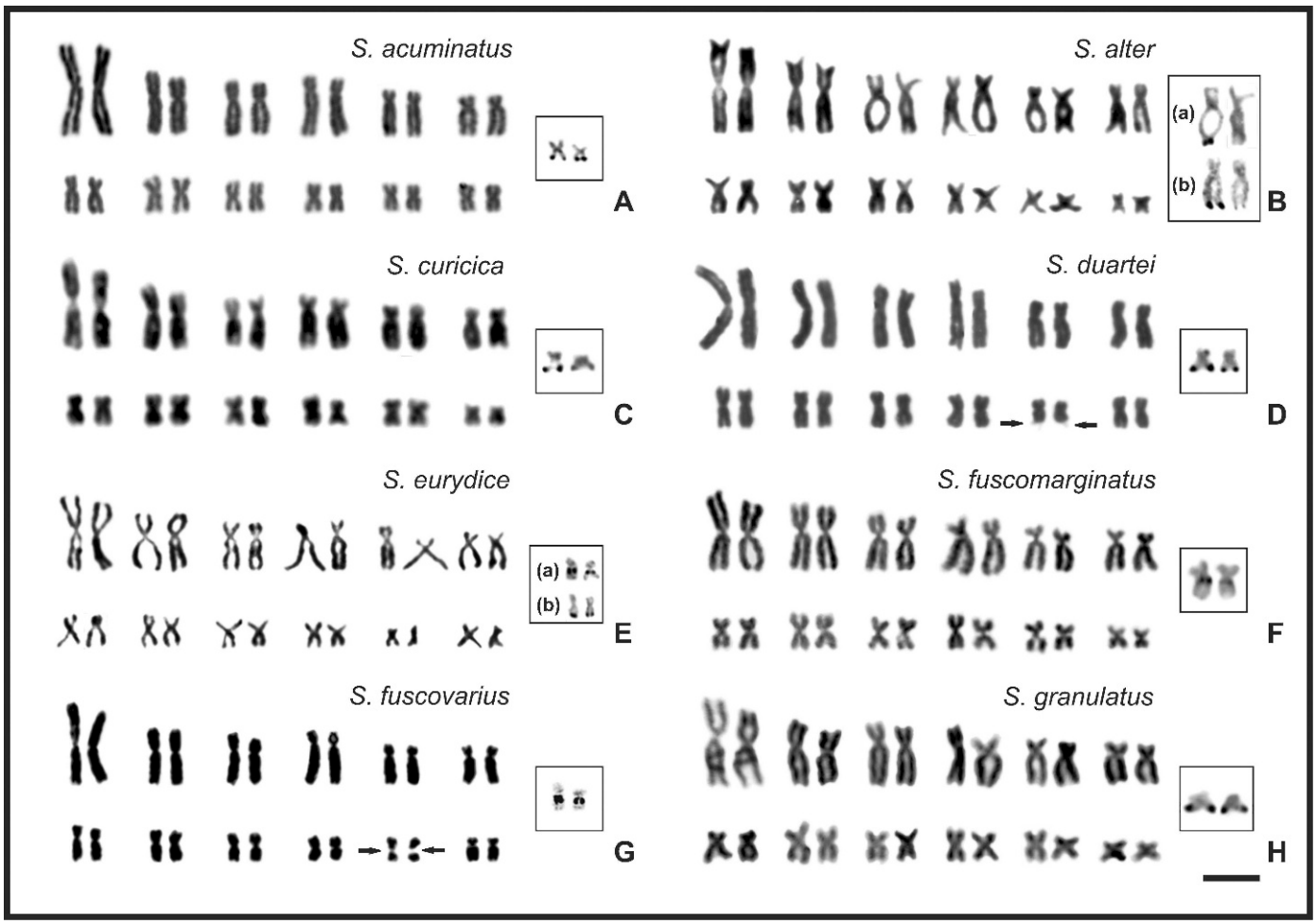

Fig. 3. Giemsa stained karyotypes and chromosomes after Ag-NOR technique (inset) of eight species of the *Scinax ruber* clade, unassigned to any group. Ag-NORs are in pair 11, at terminal region in A, C, D, E(b), and H, or at proximal region in E(a), F, and G. In B, Ag-NORs are in pair 3, but only one labeling is seen in the chromosomes in (a), which are the same as the karyogram sequentially submitted to Ag-impregnation; in (b) one of the homologues 3 showed tiny Ag-NOR. The arrows indicate the *sc*. Scale bar = 10 μm.

and 2 are *sm* in the *S. catharinae* clade and *m* in the *S. ruber* clade. Species of the latter unassigned to species group and *S. uruguayus* (*S. uruguayus* group) showed no differences in the morphology of the *m* pair 1. In the *S. catharinae* clade, the size of pairs 1 and 2 is similar (RL = 12.22 ± 0.69% and RL = 11.29 ± 0.54%, respectively), whereas in the *S. ruber* clade pair 1 is longer than pair 2 (RL = 15.27 ± 0.71% and RL = 12.29 ± 0.63%, respectively). Changes in centromere position in presumed homoeologous chromosomes of the same size are, in general, attributed to pericentric inversions. This rearrangement might explain the differences in chromosome morphology between species of the two clades. However, since pairs 1 and 2 have different size, other mechanisms might be involved, such as addition/loss of repetitive sequences or unidentified structural chromosome alterations. In this case, available C-banding provided no conclusive evidence.

The x = 12 occurs in most members of the Hylinae, including some other taxa (*Pseudis* and *Sphaenorhynchus*) of Dendropsophini, the tribe in which *Scinax* has been included. In all studied karyotypes of these hylines, pairs 1 and 2 are *m* and probably homoeologous to those of species of the *S. ruber* clade. In the karyotypes of *Dendropsophus* (2n = 30), pairs 1 and 2 are *sm* as in the *S. catharinae* clade, but probably not homoeologous to them, because they are significantly smaller and their size equivalent to pairs 3 and 4 of the hylines with 2n = 24 (Gruber et al., 2005). Thus, the *sm* morphology of pairs 1 and 2 are most likely synapomorphies of the *S. catharinae* clade.

The position of NORs provide informative variation in *Scinax*. Almost all studied species of the *S. catharinae* clade have the Ag-NORs in pair 6. Although this has not been confirmed by silver impregnation in *S. trapicheiroi*, it is suggested by the position of the secondary constrictions. Bogart (1973) considered that pair 5 presented *sc* in *S. brieni* of the *S. catharinae* clade; however, this discrepancy might be due to a different criterion of homoeology assessment, likely due to pairs 5 and 6 being very similar in size. Thus, *S. brieni* may be another species illustrating the conserved position of the NORs in the *S. catharinae* clade. The only studied species of the clade with different position of NORs is *S. canastrensis*, with terminal Ag-NORs in pair 11 like many species of the *S. ruber* clade (*S. acuminatus*, *S. curicica*, *S. duartei*, *S. granulatus*, *S. hayii*, and *S. perereca*). However, in the female of *S. canastrensis*, there was one Ag-NOR in one chromosome of pair 6. This might be an indication that in *S. canastrensis* a NOR in pair 6 still occurs, but may be inactive. In the phylogenetic hypothesis of Faivovich (2002), *S. canastrensis* is not in a particularly basal position within the *S. catharinae* clade, suggesting that the presence of NORs

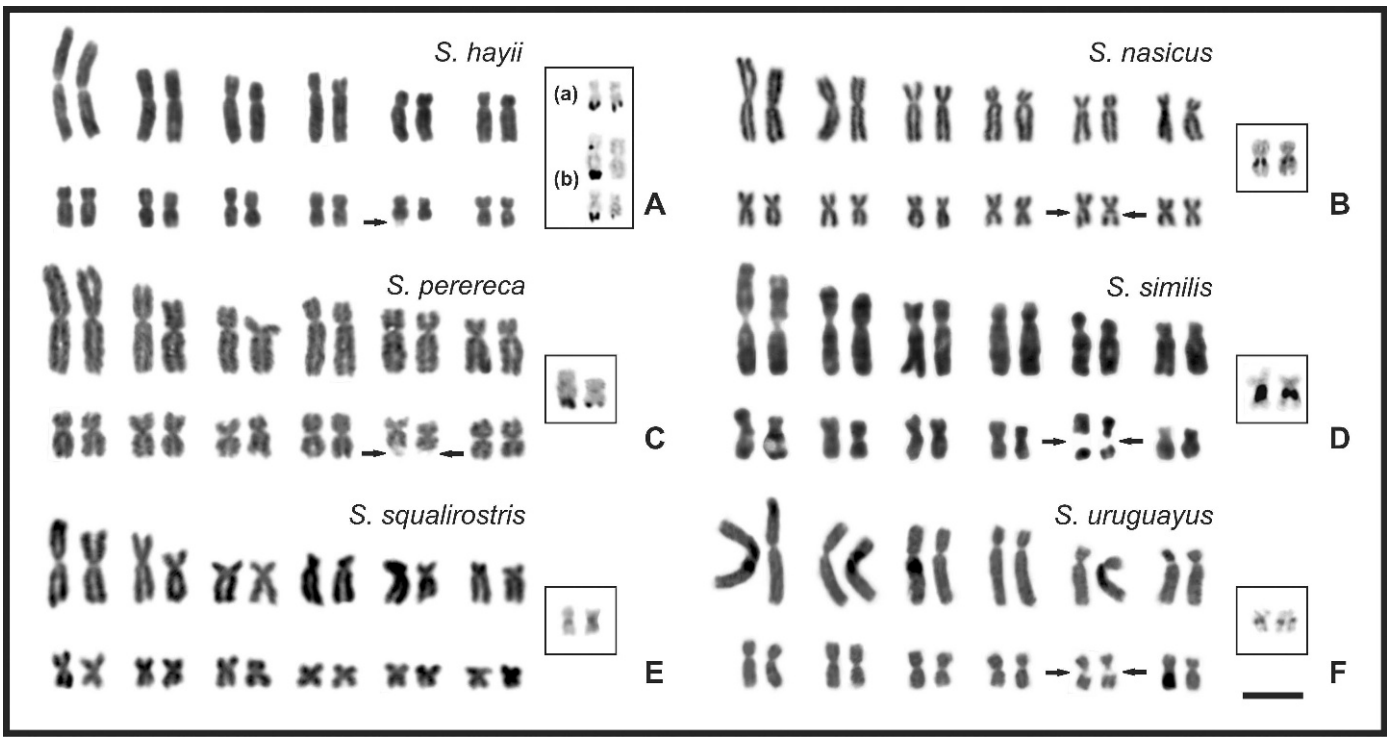

Fig. 4. Giemsa stained karyotypes and chromosomes after Ag-NOR technique (inset) of six species of the *Scinax ruber* clade. *Scinax uruguayus* belongs to the *S. uruguayus* group and the remaining species are unassigned to any group. Ag-NORs are in pair 11, at terminal region in A (a,b) and C, or at proximal region in B, D, E, and F. The chromosomes in A (b), correspond to pairs 8 and 11; one of the homologues 11 showed tiny Ag-NOR. The arrows indicate the *sc*. Scale bar = 10 μm.

at the interstitial of pair 6*q* is likely a synapomorphy of the *S. catharinae* clade, while the presence of NORs in pair 11 in *S. canastrensis* is a reversion to the ancestral state. Another Dendropsophini with NORs on pair 6*q* is *Pseudis caraya* (Busin et al., 2006), but in all other *Pseudis* species they are located on pair 7*q*, or their equivalent chromosome in *P. cardosoi* (with 2n = 2x = 28 due to fissions of first and second pairs; Busin et al., 2001, 2006, 2008). In *Dendropsophus*, the location of NORs is highly variable, with species having them alternatively on pairs 1, 7, 10, 11, 13, 14, and 15, or even multiple NORs (Medeiros et al., 2003; Gruber et al., 2005). Unfortunately, their position is unknown in *Scarthyla, Xenohyla*, and *Sphaenorhynchus*. Another possible informative character is the position of the ribosomal genes which seem to be close to the centromere in *S. argyreornatus, S. ariadne*, *S. berthae*, *S. littoralis*, *S. obtriangulatus* and *S. rizibilis*, whereas they are more interstitial on 6*p* in the other species of the clade.

Almost all studied species of the *S. ruber* clade have Ag-NORs in pair 11, with the exception of *S. alter*. Previous studies (Anderson, 1991; Baldissera et al., 1993; Pombal et al., 1995; Kasahara et al., 2003) seem to confirm this finding, even though the position of Ag-NORs has been assigned to chromosome pairs 10, 11, or 12, according to different authors. Faivovich (2002) included the position of the NORs as a character, stressing that published differences for *S. fuscovarius* (NORs in pair 10 [Anderson, 1991] vs. pair 11 [Baldissera et al., 1993]) could actually be due to problems in homoeology assessment, probably due to the general morphological similarities among pairs 10, 11, and 12. He further considered as different states the NORs in pair 12 as reported in *S. staufferi* (Anderson, 1991), *S. hayii* (Baldissera et al., 1993), and *S. perereca* (Pombal et al., 1995), and NORs in pair 10 as reported in *S. elaeochrous* (Anderson, 1991). Given the morphological similarity of these small-sized chromosome pairs, their homoeology cannot be unequivocally established, and this character needs to be re-evaluated and likely modified in future phylogenetic analyses. Similar homoeology problems as suggested by differences in the NOR-bearing pair seem to occur in some other 2n = 24 hylids of the genera *Hypsiboas*, *Bokermannohyla*, *Hyla*, *Aparasphenodon*, *Corythomanthis*, and *Itapotihyla*, allocated to the other three tribes, e.g., Cophomantini, Hylini, and Lophiohylini (Anderson, 1991; Baldissera et al., 1993; Kasahara et al., 2003; Ananias et al., 2004; Gruber et al., 2007). Comparing the BrdU replication banding patterns (Kasahara et al., 2003; Gruber et al., 2007), Ag-NOR-bearing chromosomes are homoeologous in *S. fuscovarius*, *Aparasphenodon brunoi*, *Corythomanthis greeningi, Itapotihyla langsdorffii*, *Hypsiboas crepitans*, *H. raniceps*, and in *H. albopunctatus* (the latter with 2n = 22 or 2n = 22 + B), although not always arranged in the same position in the karyograms. This may be taken as evidence that the NOR-bearing pair 11 of *S. ruber* clade is homoeologous to those seen in species of all four Hylinae tribes, and that this character state may be plesiomorphic for Hylinae. The finding of Ag-NORs in pair 3 in *S. alter* deserves further study. Recent evidence suggests that *S. alter*, *S. auratus*, *S. crospedospilus*, and *S. cuspidatus* might be related (Alves and Carvalho e Silva, 2002; Alves et al., 2004), so it is worth studying the karyotypes of these species in order to determine the location of the NORs.

The position of the Ag-NORs on pair 11*q* is variable in the *S. ruber* clade. It could either be placed proximally, as it happens in *S. fuscomarginatus*, *S. fuscovarius*, *S. nasicus*, *S. similis*, *S. squalirostris*, and *S. uruguayus*, or terminally (*S. acuminatus*, *S. curicica*, *S. duartei*, *S. granulatus*, *S. hayii*, and *S.*

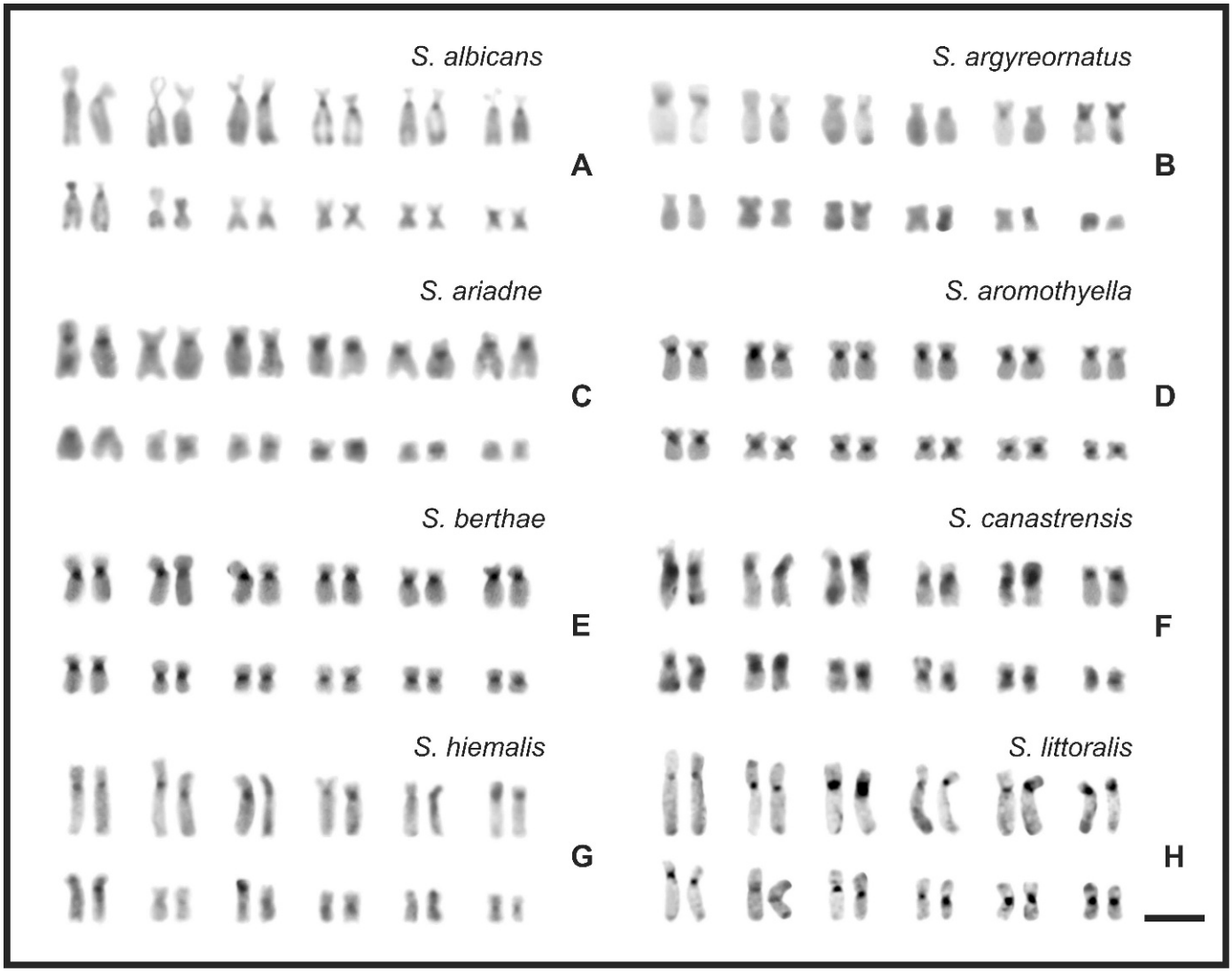

Fig. 5. C-banded karyotypes of eight species of the *Scinax catharinae* clade assigned to the *S. catharinae* group. Scale bar = 10 μm.

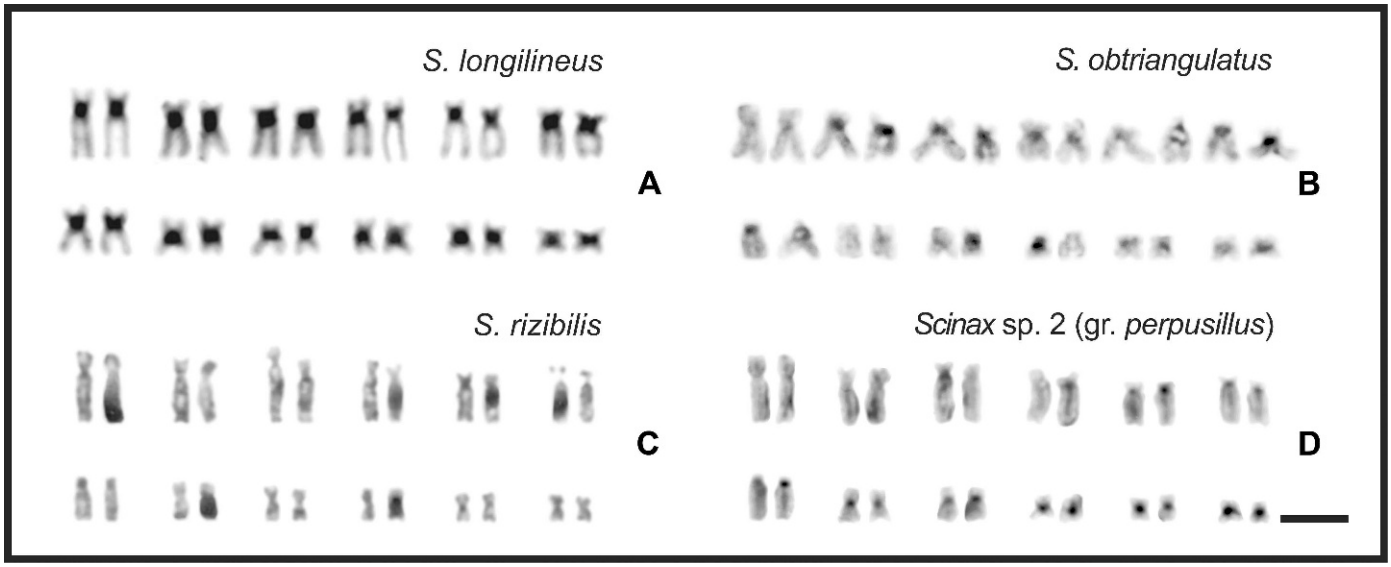

Fig. 6. C-banded karyotypes of four species of the *Scinax catharinae* clade, of which *Scinax* sp. 2 (*perpusillus* group) belongs to the *S. perpusillus* group and the remaining to the *S. catharinae* group. Scale bar = 10 μm.

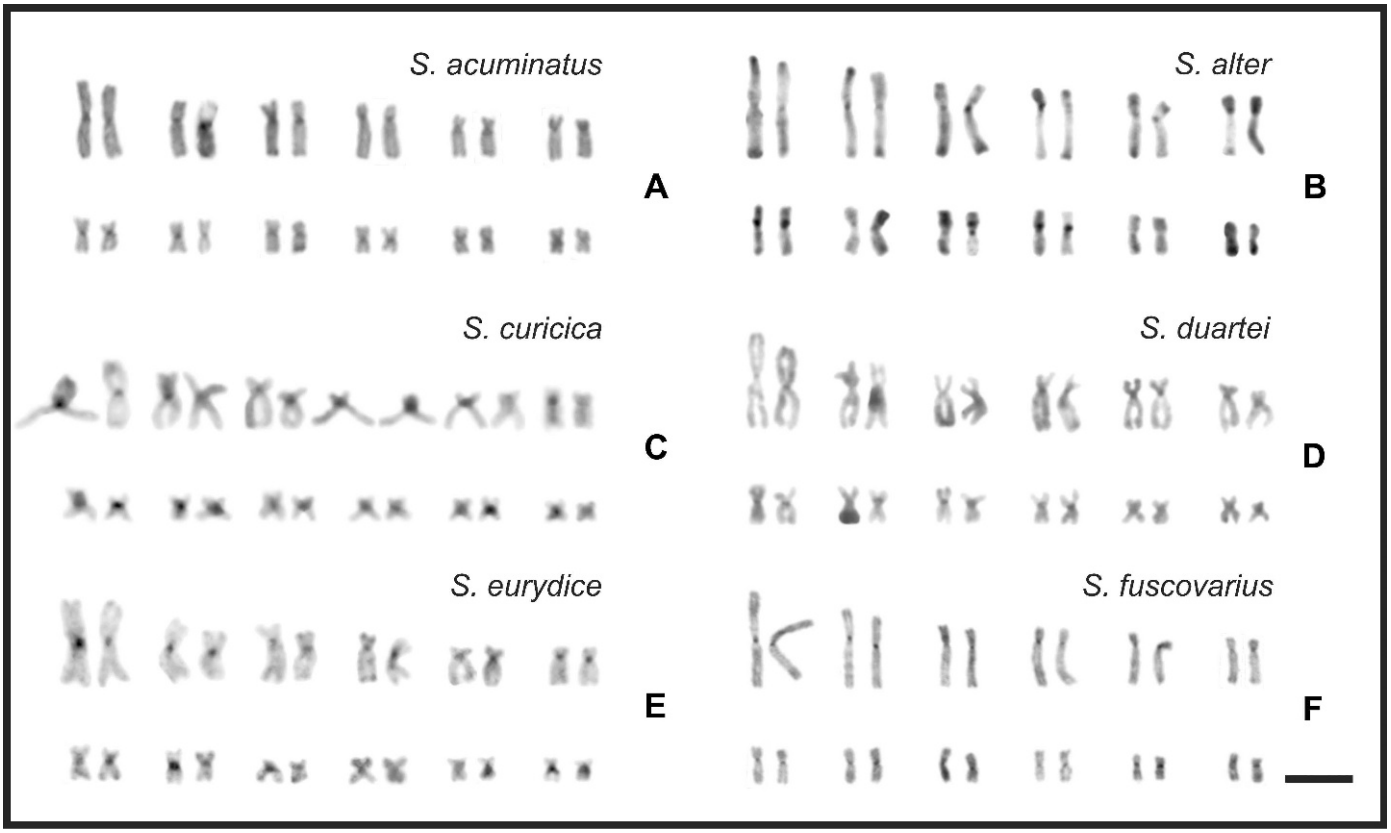

Fig. 7. C-banded karyotypes of six species of the *Scinax ruber* clade unassigned to any group. Scale bar = 10 μm.

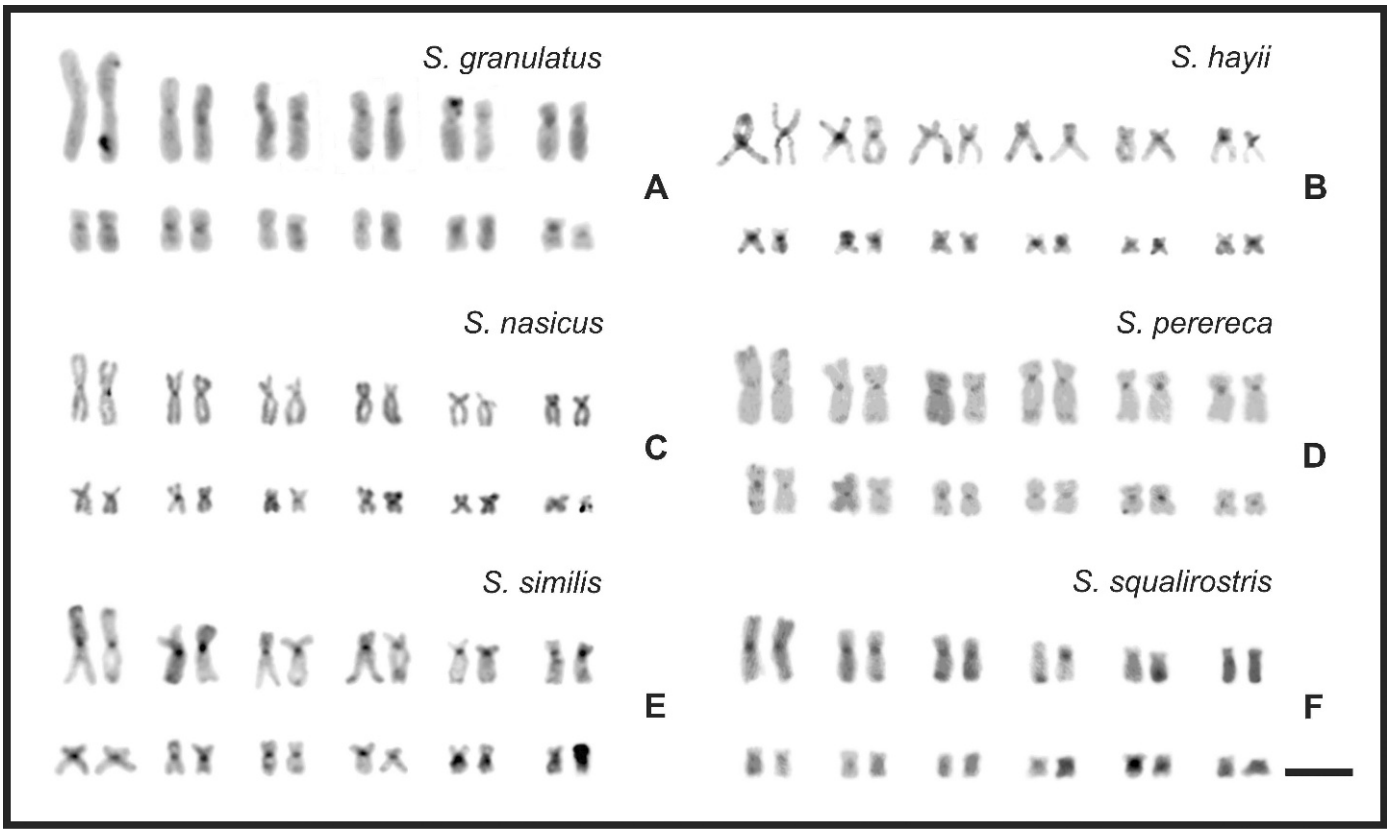

Fig. 8. C-banded karyotypes of six species of the *Scinax ruber* clade unassigned to any group. Scale bar = 10 μm.

perereca). Both positions were observed in individuals of *S. eurydice*. Variability in Ag-NOR size is relatively common in anurans (Schmid et al., 1991). While differential activity of ribosomal genes in homologous chromosomes is a possible explanation, this variation might be the consequence of different length of the rDNA segment, resulting from unequal crossing-over or exchange between sister chromatids. The two cases with a single Ag-NOR detected in some metaphases (*S. alter* and *S. hiemalis*) may represent a partial loss of the ribosomal cistrons in one of the homologues, which could be confirmed by FISH techniques using a rDNA fluorescent probe. This technique would also be useful to investigate if the additional Ag-stained sites in *S. hayii* (in chromosome 8) and in *S. canastrensis* (in chromosome 6) are actually NORs or silver stained proteins associated with a repetitive region.

The C-banding analyses revealed differences between the two major clades of *Scinax*. Species of the *S. ruber* clade showed a small centromeric labeling, whereas those of the *S. catharinae* clade have a relatively greater amount of heterochromatin with prominent C-positive bands in the centromeres, and even in the pericentromeric region in the cases of *S. aromothyella*, *S. berthae*, *S. canastrensis*, *S. littoralis*, and *S. longilineus*.

Scinax is a species-rich and taxonomically complex clade, and much remains to be learned about its karyotypic diversity and evolution. Future work should address the use of techniques that allow a suitable assessment of chromosomal homoeology, in order to enhance the number of informative karyotypic characters to study the phylogenetic relationships and chromosome evolution in this Neotropical genus.

MATERIAL EXAMINED

Scinax acuminatus: Argentina: Chaco: Departamento Chacabuco: Charata, MLP DB 2765, 2773 (males); Corrientes: Departamento Curuzú Cuatiá: El Oscuro, MLP DB 2535–8, 2542, 2544 (3 males, 3 females).
Scinax albicans: Brazil: Rio de Janeiro: Petrópolis, CFBH 10176–9 (males).
Scinax alter: Brazil: São Paulo: Ubatuba, CFBH 17293 (male); Santa Catarina: Rancho Queimado, CFBH 16733 (female).
Scinax argyreornatus: Brazil: São Paulo: Ilha do Cardoso, CFBH 17273 (juvenile), 17288–91 (3 males, 1 juvenile).
Scinax ariadne: Brazil: São Paulo: São José do Barreiro, CFBH 19543 (female).
Scinax aromothyella: Argentina: Misiones: Departamento Cainguas: Aristóbulo del Valle, Balneario del Arroyo Cuña Pirú, MLP DB 5067 (male); Parque Provincial Salto Encantado, MLP DB 3544 (male). Uruguay: Departamento Treinta y Tres: Quebrada de los Cuervos, ZVCB 14561–3 (males).
Scinax berthae: Argentina: Corrientes: Departamento Santo Tomé: Gdor. Virasoro, MLP DB 3087–8, 3093 (males); Misiones: Departamento Candelaria: Fachinal MLP DB 3908 (male); Profundidad MLP DB 2909 (male); Departamento Capital: Villa Lanús, Campus UNaM, MLP DB 3127 (male); Posadas, Barrio Villa Blosset, MLP DB 3014–5, 5068–70 (males); Departamento Iguazú: Pto. Iguazú, MLP DB 1198, 5018 (males). Brazil: São Paulo: Itirapina, unvouchered specimens (male). Uruguay: Rocha: Bañado de Santa Teresa, ZVCB 14559–60 (males).
Scinax canastrensis: Brazil: Minas Gerais: Furnas, CFBH 16729–31 (2 males, 1 female).
Scinax curicica: Brazil: Minas Gerais: Serra do Cipó, CFBH 16732 (male).
Scinax duartei: Brazil: Minas Gerais: Itamonte, CFBH 10187 (male); Rio de Janeiro: Petrópolis, CFBH 10188 (male).
Scinax eurydice: Brazil: São Paulo: Jundiaí, CFBH 16736 (male); São Jose dos Campos, CFBH 4003–5 (2 females, 1 male).
Scinax fuscomarginatus: Argentina: Corrientes: Departamento Ituzaingó: Ituzaingó, Reserva Santa Maria, MLP DB 4214–8, 4220–1, 4225, 4227, 4231–2 (9 males, 2 females).
Scinax fuscovarius: Argentina: Catamarca: Departamento Sierras El Alto, MLP DB 2667 (female); Jujuy: Departamento Manuel Belgrano: Parque Provincial Yala, Laguna Rodeo, MLP DB 4800 (male); Misiones: Departamento Cainguas: Aristóbulo del Valle, Balneario del Arroyo Cuña Pirú, MLP DB 2636, 2805 (male, female); Departamento Capital: Posadas, Barrio Villa Sarita, MLP DB 2920 (male); Villa Lanús, Campus UNaM, MLP DB 3064, 3066, 3075, 3077, 3100, 3124, 3129, 3133, 3135, (8 males, 1 female); Posadas, MLP DB 2783, 3060 (males); Departamento Gral. Manuel Belgrano: Andresito, Parque Provincial Cametti, MLP DB 1982 (female); Departamento Guaraní: El Soberbio, Refugio Tangará, MLP DB 2813 (female); Salta: Departamento Rosario de Lerma: Encón Grande, MLP DB 2557, 2559, 2563 (2 males, 1 female). Brazil: Paraná: Condoi, CFBH 5929 (male); São Paulo: Rio Claro, CFBH 5931, 16704, 17292 (1 juvenile, 2 females); Santa Maria da Serra, CFBH 3983 (male); Socorro, unvouchered specimen (male).
Scinax granulatus: Argentina: Misiones: Departamento Guaraní: Parque Provincial Moconá, MLP DB 4056, 4060, 4071 (males). Uruguay: Departamento San José: Playa Penino, MLP DB 6798 (male).
Scinax hayii: Brazil: Minas Gerais: Camanducaia, unvouchered specimens (3 males, 1 female); São Paulo: Campos do Jordão, CFBH 13648 (male).
Scinax hiemalis: Brazil: São Paulo: Jundiai, CFBH 16723–4 (males).
Scinax littoralis: Brazil: São Paulo: Ubatuba, CFBH 16734–5, 16737 (males).
Scinax longilineus (Lutz, 1968): Brazil: Minas Gerais: Poços de Caldas, 16725–6 (males).
Scinax nasicus: Argentina: Misiones: Departamento Capital: Villa Lanús, Campus UNaM, MLP DB 3062–3, 3065, 3067, 3076, 3099, 3112–4 (7 males, 2 females); Salta: Departamento Orán: Pichanal, Ruta Provincial No. 5 and San Francisco River, MLP DB 4794 (male); Santa Fe: Departamento Vera: Vera, MLP DB 2722 (male); Santiago del Estero: Departamento Gral. Taboada: Añatuya, MLP DB 2761 (male).
Scinax obtriangulatus: Brazil: São Paulo: São José do Barreiro, CFBH 19544 (male).
Scinax perereca: Argentina: Misiones: Departamento Guaraní: Parque Provincial Moconá, MLP DB 4057–9, 4061, 4070 (males); Departamento San Pedro: Colonia Victoria, MLP DB 1925 (male); Colonia Victoria, Arroyo 3 vueltas, MLP DB 1959 (male). Brazil: São Paulo: Riberão Branco, CFBH 13640–1, 1646, 13649 (males).
Scinax rizibilis: Brazil: São Paulo: Riberão Branco, CFBH 13637–9, 13642–5, 13647 (5 males, 3 females).
Scinax similis: Argentina: Corrientes: Departamento Ituzaingó: Ituzaingó, Reserva Santa Maria, MLP DB 4343 (male); Misiones: Departamento Candelaria: Campo San Juan, MLP DB 4042 (male); Departamento Capital: Villa Lanús, Campus UNaM, MLP DB 1349–51, 3140 (males); Posadas, Ruta Nacional No. 12, Km. 1329, MLP DB 3373 (male). Brazil: Minas Gerais: Três Marias, CFBH 5932–3 (females); São Paulo: Itirapina, CFBH 6567–8 (males); Rio Claro, CFBH 6587 (male).

Scinax squalirostris: Argentina: Misiones: Departamento Capital: Posadas, Barrio Villa Blosset, MLP DB 2998–9, 3001–4, 3012–3 (males); Villa Lanús, Campus UNaM, MLP DB 3047 (male). Brazil: São Paulo: Itirapina, CFBH 6566 (male). Uruguay: San José: Delta del Tigre, ZVCB 14564 (male).
Scinax trapicheiroi: Brazil: São Paulo: Ubatuba, CFBH 16703 (female).
Scinax uruguayus: Uruguay: Departamento Treinta y Tres: Quebrada de los Cuervos, ZVCB 14574–6 (males).
Scinax sp. 1 (*perpusillus* group): Brazil: Rio de Janeiro: Paratí, CFBH 16727–8 (1 male, 1 female).
Scinax sp. 2 (*perpusillus* group): Brazil: São Paulo: Ilha do Cardoso, CFBH 17274–6 (male, female, juvenile).

ACKNOWLEDGMENTS

D. Cardozo, D. Baldo, and J. Faivovich thank CONICET. D. Cardozo and D. Baldo acknowledge ANPCyT, Argentina for Grants PICT (16-35045) and PICT-O (37035). J. Faivovich thanks FAPESP procs. 05/56756-0 and 06/52088-5. J. Faivovich and D. Baldo acknowledge ANPCyT, Argentina for Grants PICT (06-223, 07-2202). DML, JFB, APZS, C. Haddad, and S. Kasahara are greatly indebted to FAPESP and CNPq for financial support. F. Kolenc and C. Borteiro acknowledge E. Zinemanas for providing working space at lab, and Agencia Nacional de Investigación e Innovación. We acknowledge C. Prado, P. Garcia, A. Miyoshi, J. Zina, F. Toledo, L. Giasson, D. Barrasso, D. Martí, M. Pereyra, S. Rosset, L. Cotichelli, C. Tomatis, M. Tedros, and C. Prigioni for providing or collaborating in the collection of some specimens, and C. Miléo and S. Gruber for help with the figures.

LITERATURE CITED

Alves, A. C. R., and S. P. Carvalho e Silva. 2002. Description of the tadpoles of *Scinax alter* and *S. cuspidatus* (Anura: Hylidae). Journal of Herpetology 36:133–137.

Alves, A. C. R., M. R. Gomes, and S. P. Carvalho e Silva. 2004. Description of the tadpole of *Scinax auratus* (Wued–Neuwied). Revista Brasileira de Zoologia 21:315–317.

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

Anderson, K. 1991. Chromosome evolution in Holarctic *Hyla* tree frogs, p. 299–328. *In*: Amphibian Cytogenetics and Evolution. D. M. Green and S. K. Sessions (eds.). Academic Press, San Diego.

Baldissera, F. A., Jr., P. S. L. Oliveira, and S. Kasahara. 1993. Cytogenetics of four Brazilian *Hyla* species (Amphibia–Anura) and description of a case with a supernumerary chromosome. Revista Brasileira de Genética 16:335–345.

Barrio, A., and D. Pistol de Rubel. 1970. Características del cariotipo de los pseudidos (Amphibia–Anura). Physis 79:505–510.

Beçak, M. L. 1968. Chromosomal analysis of eighteen species of Anura. Caryologia 21:191–208.

Bogart, J. P. 1973. Evolution of anuran karyotypes, p. 337–349. *In*: Evolutionary Biology of the Anurans. J. L. Vial (ed.). University of Missouri Press, Columbia, Missouri.

Busin, C. S., G. V. Andrade, J. Bertoldo, M. L. Del Grande, M. Uetanabaro, and S. M. Recco-Pimentel. 2008. Cytogenetic analysis of four species of *Pseudis* (Anura, Hylidae), with the description of ZZ/ZW sex chromosomes in *P. tocantins*. Genetica 133:119–127.

Busin, C. S., V. Giovani, and S. H. Recco-Pimentel. 2001. Chromosomal rearrangements of variation in the number of chromosomes in *Pseudis* (Amphibia, Anura). Genetica 110:131–141.

Busin, C. S., A. Pimentel Lima, C. Peralta De Almeida Prado, C. Strümann, S. Sequeira, Jr., and S. M. Recco-Pimentel. 2006. Chromosomal differentiation of populations of *Lysapsus limellus limellus*, *L. l. bolivianus,* and of *Lysapsus caraya* (Hylinae, Hylidae). Micron 37:355–362.

Duellman, W. E. 1967. Additional studies of chromosomes of anuran amphibians. Systematic Zoology 16:38–43.

Faivovich, J. 2002. A cladistic analysis of *Scinax* (Anura: Hylidae). Cladistics 18:367–393.

Faivovich, J., C. F. B. Haddad, P. C. A. Garcia, D. R. Frost, J. A. Campbell, and W. C. Wheeler. 2005. Systematic review of the frog family Hylidae, with special reference to Hylinae: phylogenetic analysis and taxonomic revision. Bulletin of the American Museum of Natural History 294:1–240.

Frost, D. R. 2011. Amphibian Species of the World: an Online Reference. Version 5.5. American Museum of Natural History, New York. Electronic Database accessible at http://research.amnh.org/herpetology/amphibia/

Green, D. M., and S. K. Sessions. 1991. Nomenclature for chromosomes, p. 431–432. *In*: Amphibian Cytogenetics and Evolution. D. M. Green and S. K. Sessions (eds.). Academic Press, San Diego.

Green, D. M., and S. K. Sessions. 2007. Karyology and cytogenetics, p. 2756–2841. *In*: Amphibian Biology. Vol. 7. H. Heatwole and M. Tyler (eds.). Surrey Beatty and Sons, Chipping Norton, Australia.

Gruber, S. L., C. F. B. Haddad, and S. Kasahara. 2005. Evaluating the karyotypic diversity in species of *Hyla* (Anura: Hylidae) with 2n = 30 chromosomes based on the analysis of ten species. Folia Biologica 51:68–75.

Gruber, S. L., C. F. B. Haddad, and S. Kasahara. 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.

Howell, W. M., and D. A. Black. 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia 36: 1014–1015.

Huskins, C. L. 1932. A cytological study of Vilmorin's unfixable dwarf wheat. Journal of Genetics 25:113–124.

Kasahara, S., A. P. Z. Silva, S. L. Gruber, and C. F. B. Haddad. 2003. Comparative cytogenetic analysis on four tree frog species (Anura, Hylidae, Hylinae) from Brazil. Cytogenetic and Genome Research 103:155–162.

Leviton, A. E., R. H. Gibbs, Jr., E. Heal, and C. E. Dawson. 1985. Standards in herpetology and ichthyology: part I. Standard symbolic codes for institutional resource collections in herpetology and ichthyology. Copeia 1985:802–832.

Medeiros, L. R., D. C. Rossa-Feres, and S. M. Recco-Pimentel. 2003. Chromosomal differentiation of *Hyla sanborni* (Anura, Hylidae) with a description of NOR polymorphism in *H. nana*. Journal of Heredity 94:49–154.

Nunes, R. R. A., and V. Fagundes. 2008. Cariótipos de oito espécies de anfíbios das subfamílias Hylinae e Phyllome-

dusinae (Anura, Hylidae) do Espírito Santo, Brasil. Boletim do Museu de Biologia Mello Leitão 23:21–36.

Pombal, J., Jr., C. F. B. Haddad, and S. Kasahara. 1995. A new species of *Scinax* (Anura: Hylidae) from southeastern Brazil with comments on the genus. Journal of Herpetology 29:1–6.

Rabello, M. N. 1970. Chromosomal studies in Brazilian anurans. Caryologia 23:45–59.

Rabello, M. N., M. L. Beçak, and W. Beçak. 1971. Contribuição à citotaxonomia da família Hylidae. Arquivos do Museu Nacional 54:285–286.

Reeves, A., and J. Tear. 2000. Micromeasure. Version 3.3. Avalaible at http://www.colostate.edu/Depts/Biology/MicroMeasure

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

Schmid, M., C. Steinlein, K. Kausch, and T. Haaf. 1991. Sex-determinating mechanisms and sex chromosomes in Amphibia, p. 393–430. *In*: Amphibian Cytogenetics and Evolution. D. M. Green and S. K. Sessions (eds.). Academic Press, San Diego.

Sumner, A. T. 1972. A simple technique for demonstrating centromeric heterochromatin. Experimental Cell Research 75:304–306.

White, M. J. D. 1954. Animal Cytology and Evolution. Cambridge University Press, Cambridge, U.K.