Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

Cytogenetic comparison of tree frogs of the genus *Aplastodiscus* and the *Hypsiboas faber* group (Anura, Hylidae)

K.A. Carvalho[1], P.C.A. Garcia[2] and S.M. Recco-Pimentel[1]

[1]Departamento de Anatomia, Biologia Celular e Fisiologia e Biofísica, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, SP, Brasil
[2]Departamento de Zoologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil

Corresponding author: S.M. Recco-Pimentel
E-mail: shirlei@unicamp.br

Genet. Mol. Res. 8 (4): 1498-1508 (2009)
Received September 22, 2009
Accepted October 13, 2009
Published December 18, 2009

ABSTRACT. Four species of *Aplastodiscus* and two species of *Hypsiboas* were cytogenetically compared. *Aplastodiscus perviridis*, *A. cochranae*, *H. albomarginaus*, and *H. faber* had 2n = 24 chromosomes, while *A. albosignatus* and *A. leucopygius* had 2n = 20 and 2n = 18 chromosomes, respectively. *Aplastodiscus perviridis* and *A. cochranae* had identical karyotypes, as indicated by their chromosomal morphology, the location of the nucleolus organizer region (NOR) on chromosome pair 12, and the heterochromatin pattern. The NOR-bearing chromosomes of *A. albosignatus* and *A. leucopygius* (pair 9) were very similar in size and morphology (metacentric) when compared to *A. perviridis* and *A. cochranae* (pair 12) and to *H. faber* (pair 11); the NOR of these chromosomes also occurred in the same region, suggesting that these chromosomes are homologous. Although *H. albomarginatus* differs from the other species with regard to the location of its NOR on pair 2, this species had the same diploid number and a chromosomal morphology similar to that of *A. perviridis* and *A. cochranae*. Chromosomal differentiation among the species appears to have occurred by reduction in chromosome number.

Key words: *Aplastodiscus*; *Hypsiboas*; Karyotype; Nucleolus organizer region

INTRODUCTION

The genus *Aplastodiscus* A. Lutz in B. Lutz (1950) currently consists of 15 species: *A. albofrenatus* (Lutz, 1924), *A. albosignatus* (Lutz and Lutz, 1938), *A. arildae* (Cruz and Peixoto, 1985), *A. callipygius* (Cruz and Peixoto, 1985), *A. cavicola* (Cruz and Peixoto, 1985), *A. cochranae* (Mertens, 1952), *A. ehrhardti* (Muller, 1924), *A. eugenioi* (Carvalho e Silva and Carvalho e Silva, 2005), *A. flumineus* (Cruz and Peixoto, 1985), *A. ibirapitanga* (Cruz and Peixoto, 1985), *A. leucopygius* (Cruz and Peixoto, 1985), *A. musicus* (Lutz, 1949), *A. perviridis* (Lutz and Lutz, 1950), *A. sibilatus* (Cruz et al., 2003) and *A. weygoldti* (Cruz and Peixoto, 1987) (for review, see Faivovich et al., 2005; Frost, 2009). However, until the systematic revision of the Hylidae by Faivovich et al. (2005), this genus had only two species, *A. perviridis* A. Lutz in B. Lutz (1950) and *A. cochranae* (Mertens, 1952). *Aplastodiscus perviridis* occurs from central and southeastern Brazil to northeastern Argentina (Cei and Roig, 1961; Frost, 2009), while *A. cochranae* occurs at only three locations in the State of Santa Catarina, Brazil (Garcia et al., 2001).

The genus *Aplastodiscus* was initially separated from the genus *Hyla* Laurenti (1768) because of differences in finger and toe structure (A. Lutz in B. Lutz, 1950). However, since there are close morphological similarities between these two genera, some authors have doubted the validity of this separation, and subsequent publications continued to use the name *Hyla perviridis* instead of *A. perviridis* (Bokermann, 1967; Bokermann and Sazima, 1973; Cardoso et al., 1989; Cardoso and Haddad, 1992). The species *A. cochranae* was described as *Hyla cochranae* Mertens (1952), but Bokermann (1966) considered it synonymous to *Aplastodiscus perviridis*, a proposal accepted by various authors (Lutz, 1973; Duellman, 1977; Cei, 1980; Frost, 1985; Lavilla, 1992). Garcia et al. (2001) validated *A. cochranae* based on external morphology.

According to Lutz (1950), the species *Hypsiboas albosignatus* (previously referred to as *Hyla albosignata*) is the closest to *A. perviridis* because of its size, color and song. Bokermann (1967) also reported similar song patterns for *A. perviridis*, *A. albofrenatus* (previously *Hyla albofrenata*) and *A. albosignatus* (previously *Hyla albosignata*). Another characteristic that approximates these species is the development of metacarpal and metatarsal tubercles, which could be related to the habit of digging burrows in mud (Garcia et al., 2001). A morphological phylogenetic analysis of the genus *Hyla* that included *Aplastodiscus* has corroborated the close relationship among these species (Silva, 1998).

Haddad et al. (2005) reported that *A. perviridis* had the same rare reproductive behavior, which is specific to species of the *A. albofrenatus* and *A. albosignatus* groups (referred to by Haddad as the *Hyla albofrenata* and *H. albosignata* complexes). Based on this similarity, these authors suggested a monophyletic origin for *Aplastodiscus* and these *Hyla* complexes.

Faivovich et al. (2005) provided a systematic review of the Hylidae based on molecular data. In this review, Brazilian species previously included in the "albosignata" and "albofrenata" complexes of the *Hyla albomarginata* group were transferred to the genus *Aplastodiscus*. In addition, the two species of the "albomarginata" complex of *Hyla*, e.g., *Hyla albomarginata*, were included in the *Hypsiboas faber* group. According to these authors, the genera *Aplastodiscus* and *Hypsiboas* are members of the tribe Cophomantini of Hylinae.

There have been few cytogenetic studies of *Aplastodiscus* and of the species of the *Hypsiboas faber* group, with most of the karyotypes having been described using conventional techniques. Bogart (1973) found a distinct chromosome number for *A. albofrenatus* (Tijuca, RJ, 2n = 24 and Boracéia, SP, 2n = 22) and *A. albosignatus* (Boracéia, SP, 2n = 20 and Teresópolis, RJ, 2n = 18). In the *Hypsiboas faber* group, only the karyotype of *H. albomarginatus* (2n = 24) (Beçak, 1968; Gruber et al., 2007) has been described.

In the present study, four species of *Aplastodiscus* representing the *A. perviridis* group (*A. perviridis* and *A. cochranae)* and the *A. albosignatus* group (*A. albosignatus* and *A. leucopygius*) and two species currently included in the *H. faber* group (*H. albomarginatus* and *H. faber*) were analyzed cytogenetically in order to assess the relationship between *A. perviridis* and *A. cochranae*, and between *Aplastodiscus* and the species of the *H. faber* group.

MATERIAL AND METHODS

The collection sites and the number of specimens analyzed are shown in Table 1. Permissions for collecting the specimens were issued by the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA - Proc. No. 02001.008867/01-92). All voucher specimens were deposited in the Célio Fernando Baptista Hadadd (CFBH) Collection, in the Department of Zoology of the State University of São Paulo (UNESP), Rio Claro, SP, Brazil.

Table 1. Data for the specimens analyzed.

Species	Locality (municipality/state)	Number of specimens analyzed	Voucher number in the CFBH Collection
Aplastodiscus perviridis A. Lutz and B. Lutz, 1950	Poços de Caldas (MG)	7 (6 m; 1 f)	5840; 6987-6989; 7010-7012
	São Bento do Sul (SC)	5 (m)	7406-7407; 5547-5549
Aplastodiscus cochranae Mertens, 1952	Rancho Queimado (SC)	5 (m)	6991; 7001; 7003-7005
Aplastodiscus albosignatus A. Lutz and B. Lutz, 1938	Piraquara (PR)	1 (m)	5546
	São Bento do Sul (SC)	5 (m)	5543-5545; 6992-6993
Aplastodiscus leucopygius Cruz and Peixoto, 1985	Mogi das Cruzes (SP)	7 (m)	4012-4013; 6646-6647; 7391-7393
	Maricá (RJ)	2 (m)	7395-7396
Hypsiboas albomarginatus Spix, 1824	Bertioga (SP)	5 (m)	6406-6410
	Picinguaba (SP)	4 (m)	7408-7410; 4011
	Mogi das Cruzes (SP)	2 (m)	6403-6404
Hypsiboas faber Wied-Neuwied, 1821	Mogi das Cruzes (SP)	2 (m)	4014; 6650
	Biritiba Mirim (SP)	1 (m)	6982

CFBH = Collection of the Department of Zoology of the State University of São Paulo (UNESP), Rio Claro, SP, Brazil. f = female, m = male.

Mitotic metaphases were obtained according to King and Rofe (1976) and Schimid (1978). The chromosomes were stained with 10% Giemsa in phosphate-buffered saline, pH 6.8. C-banding and Ag-NOR (nucleolus organizer region) techniques were carried out according to Sumner (1972) and Howell and Black (1980), respectively. Ag-NOR locations were confirmed with fluorescent *in situ* hybridization (Viégas-Péquignot, 1992) using a 28S rDNA probe of the recombinant plasmid HM123, which contains a fragment of rDNA of *Xenopus laevis* (Meunier-Rotival et al., 1979). The preparations were examined using an Olympus BX60 light microscope. The chromosomes were classified according to Green and Sessions (1991).

RESULTS

All the specimens of *A. cochranae*, *A. perviridis*, *H. albomarginatus*, and *H. faber* had a diploid number of 2n = 24 chromosomes, whereas *A. albosignatus* had 2n = 20 and *A. leucopygius*, 2n = 18. The chromosomal morphology was similar in all of these species, with pairs 1, 2, 9, 10, 11, and 12 being metacentric, and pairs 4, 5, 6, and 7 being submetacentric. There

was some variation in pair 3, which was metacentric in *H. albomarginatus* and submetacentric in the other species; similarly, pair 8 was submetacentric in *A. albosignatus* and *A. leucopygius* and metacentric in the other species (Figures 1A-F and 4A-F, Table 2). Heterochromatin was detected in *A. cochranae*, *A. perviridis* and *H. albomarginatus*. Heterochromatin blocks were observed in the centromeric regions of all chromosomes, but differed in amount. In *Aplastodiscus cochranae* and *A. perviridis*, heterochromatic blocks were detected in the telomeric region of the short arms of pairs 1 and 2 and in the long arms of pairs 1 to 8, as well as interstitially in the long arms of pairs 6, 10, 11, and 12, and in the short arms of pair 11 of both species. Pair 12 had heterochromatin adjacent to the NOR (Figures 2A-C and 4A, B,E). In *H. albomarginatus*, positive C-bands were found in the telomeric regions of the short arms of pairs 1, 2 and 3 and in the long arms of pairs 2 to 9. Interstitial heterochromatin was seen in the short arms of pairs 2 to 4 and in the long arms of pairs 5 and 6. On chromosome 2, the heterochromatin band coincided with the NOR and extended from this region to the centromere (Figures 2A-C and 4A,B,E).

The NOR occurred in the telomeric region of the long arm of pair 12 of *A. cochranae* and *A. perviridis* and of pair 9 of *A. albosignatus* and *A. leucopygius*. In *H. albomarginatus*, the NOR was located interstitially in the short arms of pair 2, and was heteromorphic for the homologous chromosomes. *Hypsiboas faber* had an NOR in the telomeric region of the long arm of pair 11 (Figures 3A-F and 4A-F). Secondary constrictions were frequently seen in some chromosomes of all species and were always associated with the NOR. In all species, the Ag-NOR coincided with the fluorescent *in situ* hybridization labeling obtained with the rDNA probe (Figure 3A-F). Table 3 summarizes the cytogenetic data for *Aplastodiscus* and the *Hypsiboas* species.

Table 2. Morphometrical data for chromosomes of *Aplastodiscus* and *Hypsiboas* species.

	Chromosomes											
	1	2	3	4	5	6	7	8	9	10	11	12
A. perviridis												
RL%	14.9	12.7	11.0	10.3	8.7	7.1	6.4	5.8	5.6	5.1	4.6	3.5
CI	0.45	0.41	0.31	0.31	0.35	0.27	0.33	0.41	0.43	0.44	0.40	0.42
CC	M	M	SM	SM	SM	SM	SM	M	M	M	M	M
A. cochranae												
RL%	14.7	12.5	10.6	10.0	8.8	7.2	6.5	5.8	5.6	5.3	4.9	4.8
CI	0.46	0.44	0.27	0.35	0.34	0.30	0.37	0.45	0.45	0.46	0.45	0.48
CC	M	M	SM	SM	SM	SM	SM	M	M	M	M	M
A. albosignatus												
RL%	15.9	13.0	11.3	10.9	10.3	10.0	8.7	6.6	4.9	2.2		
CI	0.46	0.41	0.33	0.31	0.29	0.29	0.29	0.27	0.40	0.45		
CC	M	M	SM	SM	SM	SM	SM	SM	M	M		
A. leucopygius												
RL%	17.9	13.9	11.5	11.2	10.9	10.2	8.6	6.4	2.9			
CI	0.48	0.39	0.31	0.31	0.32	0.30	0.30	0.29	0.43			
CC	M	M	SM	SM	SM	SM	SM	SM	M			
H. albomarginatus												
RL%	15.3	15.1 13.2*	11.7	10.5	9.3	8.3	6.6	5.8	5.5	5.1	4.1	3.6
CI	0.48	0.41 0.40*	0.41	0.33	0.30	0.28	0.40	0.41	0.44	0.47	0.44	0.46
CC	M	M	M	SM	SM	SM	M	M	M	M	M	M
H. faber												
RL%	15.0	12.6	10.9	10.3	8.8	7.1	6.4	5.7	5.5	5.1	4.6	4.0
CI	0.45	0.41	0.30	0.34	0.35	0.28	0.32	0.46	0.42	0.44	0.40	0.44
CC	M	M	SM	SM	SM	SM	SM	M	M	M	M	M

RL = relative length; CI = centromeric index; CC = centromeric classification; M = metacentric; SM = submetacentric. (* = values for the morphs of chromosome 2 of *H. albomarginatus*).

Figure 1. Karyotypes of *Aplastodiscus perviridis* (A), *A. cochranae* (B), *A. albosignatus* (C), *A. leucopygius* (D), *Hypsiboas albomarginatus* (E), and *H. faber* (F). Arrows indicate the secondary constrictions. Bar = 10 µm.

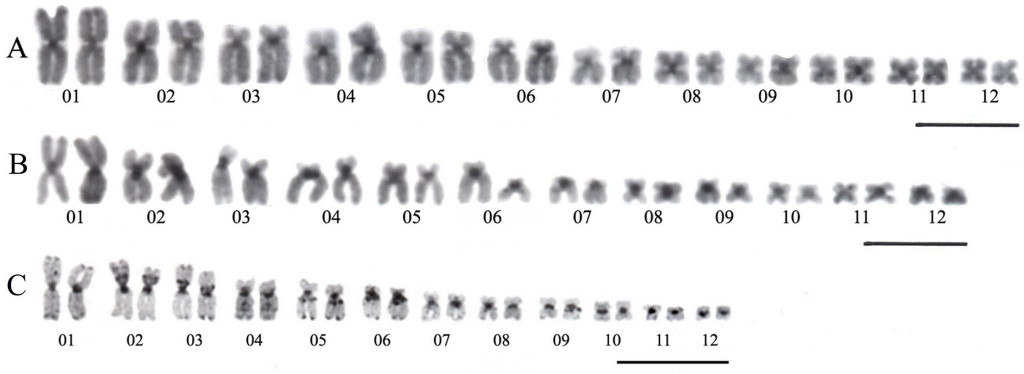

Figure 2. Karyotypes of *Aplastodiscus perviridis* (A), *A. cochranae* (B) and *Hypsiboas albomarginatus* (C) stained by C-banding. Arrows indicate interstitial and telomeric bands. Bar = 10 µm.

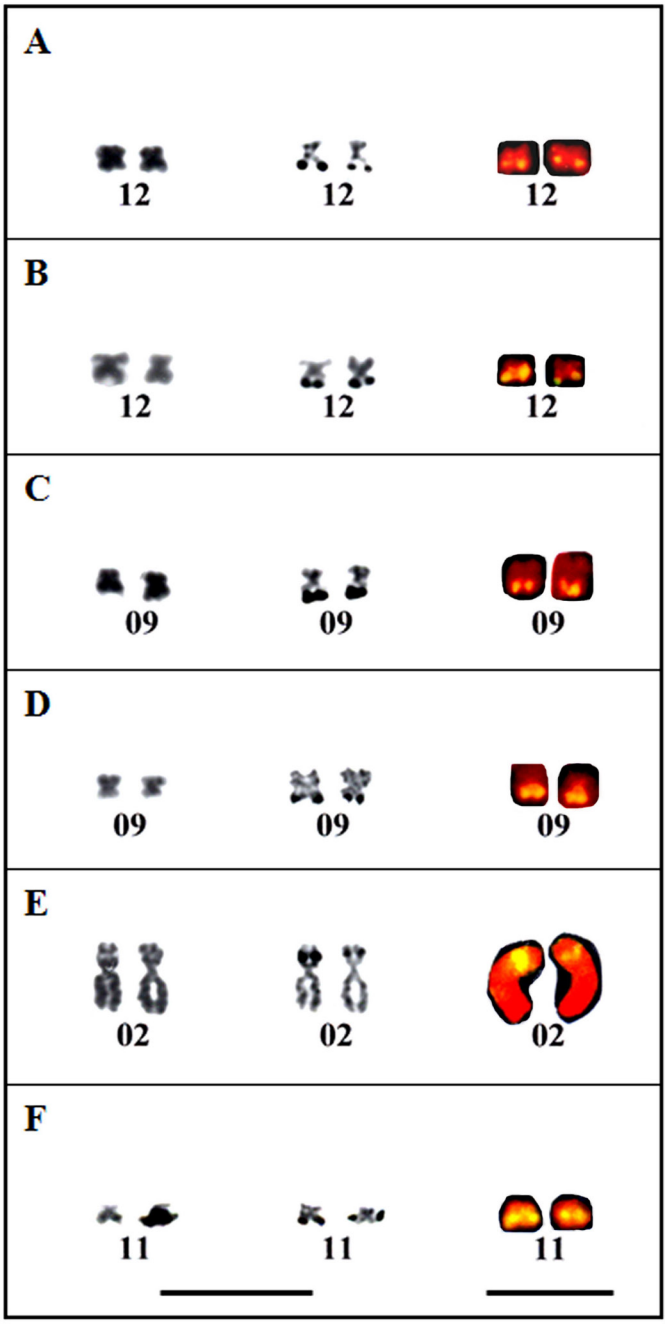

Figure 3. Chromosomal pairs containing the NOR region, as detected by Giemsa staining, silver staining and *in situ* hybridization with rDNA probe. *Aplastodiscus perviridis* (A), *A. cochranae* (B), *A. albosignatus* (C), *A. leucopygius* (D), *Hypsiboas albomarginatus* (E), and *H. faber* (F). Bar = 10 µm.

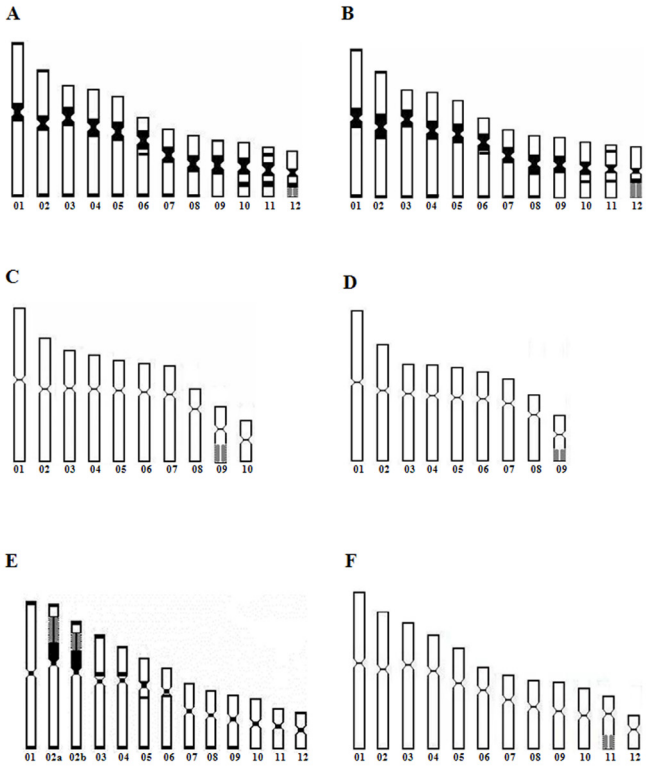

Figure 4. Ideograms of the karyotypes of *Aplastodiscus perviridis* (A), *A. cochranae* (B), *A. albosignatus* (C), *A. leucopygius* (D), *Hypsiboas albomarginatus* (E), and *H. faber* (F). The black blocks indicate C bands and the gray areas show the NOR. The hatched regions are the secondary constrictions. In *E*, the letters *a* and *b* indicate the morphs of chromosome 2.

Table 3. Cytogenetic data for species of *Aplastodiscus* and *Hypsiboas*.

Old taxon	Current taxon (sensu Faivovich et al., 2005)	2n	Chromosomal morphology	NOR-bearing pair	Heterochromatin (non-centromeric C-bands)	
					Interstitial	Pericentromeric
Aplastodiscus perviridis	*Aplastodiscus perviridis*	24	M; M; SM; SM; SM; SM; SM; M; M; M; M; M	12q12q	11p, 11q and 10q	pair 6
Aplastodiscus cochranae	*Aplastodiscus cochranae*	24	M; M; SM; SM; SM; SM; SM; M; M; M; M; M	12q12q	11p and 10q	pair 6
Hyla albomarginata	*Hypsiboas albomarginatus*	24	M; M; M; SM; SM; SM; M; M; M; M; M; M	2p2p	2p, 3p, 4p and 5 q, 6q	pair 2
Hyla faber	*Hypsiboas faber*	24	M; M; SM; SM; SM; SM; SM; M; M; M; M; M	11q11q	-	-
Hyla albosignata	*Aplastodiscus albosignatus*	20	M; M; SM; SM; SM; SM; SM; SM; M; M	9q9q	-	-
Hyla leucopygia	*Aplastodiscus leucopygius*	18	M; M; SM; SM; SM; SM; SM; SM; M	9q9q	-	-

p = short arm; q = long arm.

DISCUSSION

The analysis of chromosomal number and morphology has been useful for differentiating some frog species that have a similar external morphology, e.g., *Megaelosia* spp (Giaretta and Aguiar-Jr, 1998; Rosa et al., 2003), *Dendropsophus nanus* and *D. sanborni* (as *Hyla* in Medeiros et al., 2003) and *Colostethus* (Veiga-Menoncello et al., 2003). As shown here, the karyotypes of *A. cochranae* and *A. perviridis* had the same diploid number of 24 chromosomes and a conserved chromosomal morphology. This characteristic is frequent in the Hylidae, as reported for closely related species such as *H. marginatus*, *H. semiguttatus* and *Hypsiboas* sp (aff. *H. semiguttatus*) (Ananias et al., 2004), *H. bischoffi* and *H. guentheri* (Raber et al., 2004), *H. polytenius* and *H. leptolineatus* (Vieira, 2004). However, in these species, the karyotypes could be distinguished by the NOR location and/or heterochromatin pattern. In contrast, in addition to having the same chromosomal number and morphology shown here, the NOR location and heterochromatin pattern were also identical in *A. cochranae* and *A. perviridis*, making it impossible to distinguish these two species based solely on their cytogenetic characteristics.

Although the two species of the *A. albosignatus* group, *A. albosignatus* and *A. leucopygius*, differed in their chromosomal number (2n = 20 and 18, respectively), their chromosomal morphology was very similar. The difference in chromosomal number apparently resulted from the loss of a small metacentric chromosome, probably chromosome 10, since chromosome pair 9 in the two species had almost the same size and had the NOR in the same position. Other chromosomes in these species were also morphologically very similar. The results for *A. albosignatus* differed from those reported by Bogart (1973), who analyzed two other populations assigned to this species, i.e., Boracéia, SP, with 2n = 20 and Teresópolis, RJ, with 2n = 18 chromosomes. The karyotype with 2n = 18 chromosomes found by Bogart is identical to that of *A. leucopygius* analyzed here and collected from a region (Maricá) very close (~50 km) to Teresópolis. Since *A. leucopygius* from the same locality (Teresópolis, RJ) was described by Cruz and Peixoto (1984), the cytogenetic data suggest that the species studied by Bogart (1973) was, in fact, *A. leucopygius* and not *A. albosignatus.*

The karyotypes of *A. albosignatus* (2n = 20) and *A. leucopygius* (2n = 18) were also similar to that of *A. cochranae* and *A. perviridis* (2n = 24). The difference between these species appears to have resulted from a reduction in the number of chromosomes, since similar karyotypes, with 2n = 24, occur in other Hylinae species. This reduction involves mainly the smaller chromosomes, since the first seven chromosomes are highly conserved in size and morphology. Also, the NOR-bearing chromosome 9 in *A. albosignatus* and *A. leucopygius* is very similar in size, morphology (metacentric) and NOR location (telomeric region) to pair 12 of *A. cochranae* and *A. perviridis*. A reduction in chromosome number has also been used to explain similar karyotypic variations in other anurans (Beçak, 1968; Bogart, 1970; Veiga-Menoncello et al., 2003; Siqueira-Jr. et al., 2004; Gruber et al., 2007). Although the chromosomal morphology of *H. albomarginatus* was very similar to that of the other species studied here, the location of the NOR on the large chromosome pair 2 differentiated this species from the others.

The NOR heteromorphism seen in the homologous chromosomes of pair 2 in *H. albomarginatus* is rather common in anurans and probably resulted from the amplification of rDNA sequences, which could explain the small increase in total length of the larger NOR-bearing chromosome.

The molecular data reported by Faivovich et al. (2005) showed that *H. albomarginatus* (previously *Hyla albormarginata*) was more closely related to other Hylinae species and was

included in the *H. faber* group, whereas *A. albosignatus* and *A. leucopygius* (previously *Hyla albosignata* and *Hyla leucopygia* of the *Hyla albormarginata* group) were related to *Aplastodiscus*. Both genera are in the same monophyletic tribe within the Hylinae. The karyotype and the NOR-bearing chromosomal pair in *H. faber* are very similar to those of *A. perviridis* and *A. cochranae*, suggesting a close relationship, whereas in *H. albomarginatus*, the NOR location (pair 2) and the heterochromatin pattern are very distinct. The NOR in pair 11 of *H. faber* also occurs at the same site in *H. raniceps* (*H. albopunctata* group), whereas in *H. crepitans* (*H. faber* group) (Gruber et al., 2007) the NOR occurs at a site located interstitially. These findings suggest that the NOR sites and heterochromatin dispersion have been involved in the chromosomal evolution of this group.

The cytogenetic data obtained so far indicate that the species of *Aplastodiscus* and the *H. faber* group are very closely related and may have originated from a common ancestor. The chromosomal differentiation of the species analyzed here appears to have occurred by a reduction in the chromosome number (from 2n = 24 to 2n = 20 and 2n = 18), as already postulated by Gruber et al. (2007) to explain the reduction from 2n = 24 to 2n = 22 in *H. albopunctatus*, as well as by rearrangements involving mainly the smaller chromosomes (including the NOR-bearing ones). The latter conclusion is supported by the finding that the size and morphology of the first seven chromosomes are highly conserved in both genera.

ACKNOWLEDGMENTS

The authors thank Denise Maria Peccinini-Seale (deceased), Marilia T. Hartmann, Fernando Ananias, Sérgio Siqueira Junior, Samantha Celi Vieira, and Marcos Yamamoto for collecting some of the specimens. Research supported by the Brazilian agencies Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, grant #04/10901-6) to S.M. Recco-Pimentel and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, grant #481124/2004-7).

REFERENCES

Ananias F, Garcia PC and Recco-Pimentel SM (2004). Conserved karyotypes in the *Hyla pulchella* species group (Anura, Hylidae). *Hereditas* 140: 42-48.

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

Bogart JP (1970). Systematic problems in the amphibian family Leptodactylidae (Anura) as indicated by karyotypic analysis. *Cytogenetics* 9: 369-383.

Bogart JP (1973). Evolution of Anuran Karyotypes. In: Evolutionary Biology of Anurans (Vial JL, ed.). University of Missouri Press, Columbia, 337-349.

Bokermann WCA (1966). Lista Anotada das Localidades tipo de Anfíbios Brasileiros. Serviço de Documentação - USP, São Paulo.

Bokermann WCA (1967). Notas sobre cantos nupciais de anfíbios brasileiros. I (Anura). *An. Acad. Bras. Cienc.* 39: 441-443.

Bokermann WCA and Sazima I (1973). Anfíbios da Serra do Cipó, Minas Gerais, Brasil. 1: Duas espécies novas de *Hyla* (Anura, Hylidae). *Rev. Bras. Biol.* 33: 457-472.

Cardoso AJ and Haddad CFB (1992). Diversidade e turno de vocalizações de anuros em comunidade neotropical. *Acta Zool. Lilloana* 41: 93-105.

Cardoso AJ, Andrade GV and Haddad CFB (1989). Distribuição espacial em comunidades de anfíbios (Anura) no sudeste do Brasil. *Rev. Bras. Biol.* 49: 241-249.

Carvalho e Silva APT and Carvalho e Silva SP (2005). New species of the *Hyla albofrenata* group, from the States of Rio de Janeiro and São Paulo, Brazil (Anura, Hylidae). *J. Herpetol.* 39: 73-81.

Cei JM (1980). Amphibians of Argentina. *Monitore Zool. Italiano (Nuova Serie)* 2: XII+609.

Cei JM and Roig VG (1961). Batracios recolectados por la Expedición Biológica "Erspamer" a la mesopotamia argentina y selva oriental de Misiones. *Notas Biol. Fac. Cienc. Exactas*, *Fis. Nat.*, *Nordeste Corrientes*, *Zool.* 1: 5-40.

Cruz CAG and Peixoto AL (1984). Espécies verdes de Hyla: o complexo "Albosignata" (Amphibia, Anura, Hylidae). *Arq. Univ. Fed. Rur. Rio de Janeiro* 7: 31-47.

Cruz CAG and Peixoto AL (1985). Espécies verdes de *Hyla*: o complexo "albofrenata" (Amphibia, Anura, Hylidae). *Arq. Univ. Fed. Rur. Rio de Janeiro* 8: 59-70.

Cruz CAG, Pimenta BVS and Silvano DL (2003). Duas novas espécies pertencentes ao complexo de *Hyla albosignata* Lutz and Lutz, 1938, do leste do Brasil (Amphibia, Anura, Hylidae). *Bol. Mus. Nac.*, *N. S.*, *Zool.* 503: 1-13.

Duellman WE (1977). Liste der rezenten Amphibien und Reptilien: Hylidae, Centrolenidae, Pseudidae (Das Tierreich). de Gruyter, Berlin, New York.

Faivovich J, Haddad CFB, Garcia PCA, Frost DR, et al. (2005). Systematic review of the frog family Hylidae, with special reference to the Hylinae: phylogenetic analysis and taxonomic revision. *Bull. Am. Mus. Nat. Hist.* 294: 1-240.

Frost DR (1985). Amphibian Species of the World. Association of Systematics Collections and Allen Press, Lawrence, 110-203.

Frost DR (2009). Amphibian Species of the World, an Online Reference. Version 5.3. American Museum of Natural History, New York. Available at [http://research.amnh.org/herpetology/amphibia/index.html]. Accessed February 12, 2009.

Garcia PCA, Caramaschi U and Kwet A (2001). The taxonomic status of the *Hyla cochranae* Mertens and recharacterization of *Aplastodiscus* A. Lutz (Anura, Hylidae). [O status taxonômico de *Hyla cochranae* Mertens e recaracterização de *Aplastodiscus* A. Lutz (Anura, Hylidae)]. *Rev. Bras. Zool.* 18: 1197-1218.

Giaretta AA and Aguiar O Jr (1998). A new species of *Megaelosia* from the Mantiqueira Range, Southeastern Brazil. *J. Herpetol.* 32: 80-83.

Green MD and Sessions SK (1991). Nomenclature for Chromosomes. In: Amphibian Cytogenetics and Evolution (Green MD and Sessions SK, eds.). Academic Press, San Diego, 431-432.

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

Haddad CFB, Faivovich J and Garcia PCA (2005). The specialized reproductive mode of the tree frog *Aplastodiscus perviridis* (Anura: Hylidae). *Amphibia-Reptilia* 26: 87-92.

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

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

Lavilla EC (1992). Tipos portadores de nombre y localidades tipos de anfibios de Argentina. *Acta Zool. Lilloana* 42: 1-7.

Lutz B (1949). Anfibios anuros da coleção Adolpho Lutz II. Espécies verdes do gênero *Hyla* do leste meridional do Brasil. *Mem. Inst. Oswaldo Cruz* 46: 551-577.

Lutz B (1950). Hylidae in the Adolpho Lutz collection of the Instituto Oswaldo Cruz: V. Mode of locomotion and structure of hand and foot; Va. *Phyllomedusa* (Pithecopus) *burmeisteri distincta* A. Lutz: Vb. *Aplastodiscus perviridis* A. Lutz [Anfibios Anuros da coleção Adolpho Lutz: V. locomoção e estrutura das extremidades Va. *Phyllomedusa* (P.) *burmeisteri distincta* A. lutz: Vb. *Aplastodiscus perviridis* A. Lutz]. *Mem. Inst. Oswaldo Cruz* 48: 599-637.

Lutz B (1973). Brazilian Species of *Hyla*. University of Texas Press, Austin.

Lutz A and Lutz B (1950). Anfibios anuros da coleção Adolpho Lutz do Instituto Oswaldo Cruz. *Mem. Inst. Oswaldo Cruz* 48: 612-637.

Medeiros LR, Rossa-Feres DC and Recco-Pimentel SM (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.

Mertens R (1952). Eine neue *Hyla* aus Santa Catharina, Brasilien. *Senckenbergiana* 33: 165-167.

Meunier-Rotival M, Cortadas J, Macaya G and Bernardi G (1979). Isolation and organization of calf ribosomal DNA. *Nucleic Acids Res.* 6: 2109-2123.

Raber SC, Carvalho KA, Garcia PCA, Vinciprova G, et al. (2004). Chromosomal characterization of *Hyla bischoffi bischoffi* and *Hyla guentheri* (Anura, Hylidae). *Phyllomedusa* 3: 43-49.

Rosa C, Aguiar-Jr O, Giaretta AA and Recco-Pimentel SM (2003). Karyotypic variation in the genus *Megaelosia* (Anura, Hylodinae) with the first description of a B-chromosome in a leptodactylid frog. *Copeia* 1: 166-174.

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

Silva HR (1998). Phylogenetic Relationships of the Family Hylidae with Emphasis on the Relationships within the Subfamily Hylinae (Amphibia: Anura). Doctoral thesis, Museu Nacional do Rio de Janeiro, Universidade do Rio de

Janeiro, Rio de Janeiro.
Siqueira-Jr S, Ananias F and Recco-Pimentel SM (2004). Cytogenetics of three Brazilian species of *Eleutherodactylus* (Anura, Leptodactylidae) with 22 chromosomes and re-analysis of multiple translocations in *E. binotatus*. *Genet. Mol. Biol.* 27: 363-372.
Sumner AT (1972). A simple technique for demonstrating centromeric heterochromatin. *Exp. Cell Res.* 75: 304-306.
Veiga-Menoncello ACP, Lima AP and Recco-Pimentel SM (2003). Cytogenetic analysis of four central Amazonian species of *Colostethus* (Anura - Dendrobatidae) with a diploid complement of 22 chromosomes. *Hereditas* 139: 189-198.
Viégas-Péquignot E (1992). *In situ* Hybridization to Chromosomes with Biotinylated Probes. In: *In situ* Hybridization: a Practical Approach (Willernson D, ed.). Oxford University Press-IRL Press, Oxford, 137-158.
Vieira SC (2004). Caracterização Cromossômica de Espécies do Grupo de *Hyla polytaenia* (Anura, Hylidae). Master's dissertation, Instituto de Biologia, Universidade Estadual de Campinas, Campinas.