Cytogenetic and Genome Research

Original Article

Cytogenet Genome Res 2020;160:704–710
DOI: 10.1159/000512816

Received: August 13, 2020
Accepted: November 5, 2020
Published online: January 26, 2021

Cytogenetics of Four Species of the Green Clade *Aplastodiscus* Lutz, 1950 (Anura: Cophomantinae): New Insights into the Chromosomal Evolution of the Genus

Késsia L. Souza[a] Marco A. Peixoto[b] Cynthia A.V. Barreto[a] Renato N. Feio[c] Jorge A. Dergam[a]

[a]Laboratório de Sistemática Molecular (Beagle), Departamento de Biologia Animal, Universidade Federal de Viçosa, Viçosa, Brazil; [b]Laboratório de Biometria, Departamento de Biologia Geral, Universidade Federal de Viçosa, Viçosa, Brazil; [c]Museu de Zoologia João Moojen (MZUFV), Departamento de Biologia Animal, Universidade Federal de Viçosa, Viçosa, Brazil

Keywords
Chromosomal rearrangements · Evolutionary chromosomal trends · FISH · Karyotypes · Molecular cytogenetic technique

Abstract
The tree frog *Aplastodiscus* is a Neotropical taxon that encompasses 15 species in the Atlantic forest biome, with one isolated species in the Central Brazilian Cerrado. To date, only 8 species have been karyotyped, showing high levels of diploid number variation, which allowed clustering species in chromosome number groups: 2n = 24 (*Aplastodiscus perviridis* group), 2n = 22 (*Aplastodiscus albofrenatus* group), 2n = 20, and 2n = 18 (both within *Aplastodiscus albosignatus* group). This study aims to report karyotypic information on 4 species from the last 2 groups using classical and molecular cytogenetic techniques and hypothesize chromosomal evolutionary trends within the species groups. *Aplastodiscus weygoldti* showed 2n = 22; Ag-NOR and FISH 18S rDNA signals were located in the interstitial region of the short arms of chromosome pair 6. *Aplastodiscus cavicola, Aplastodiscus* sp. 4, and *Aplastodiscus* sp. 6 showed 2n = 18; Ag-NOR and FISH 18S rDNA bands were located in the terminal region of the long arm of chromosome pair 9. Our results support multiple and independent chromosome fusion events within *Aplastodiscus*, including a new chromosome fission event. Ag-NOR and FISH 18S rDNA patterns were restricted to the small chromosome pairs, similar to the other species within this genus, and confirm overall chromosome morphology conservation among the genera of Cophomantinae.

Introduction

The tree frog genus *Aplastodiscus* Lutz, 1950, informally called "green clade," belongs to the subfamily Cophomantinae. This Neotropical genus is mainly distributed in the Brazilian Atlantic Forest, in Northeastern Brazil, whereas one species reaches the riparian forests in the Cerrado biome. *Aplastodiscus* includes 15 species arranged in 4 monophyletic species groups: *A. perviridis*, *A. albosignatus*, *A. albofrenatus*, and *A. sibilatus*, supported by morphological characters, bioacoustics, molecular data, and more recently, cytogenetic characters [Cruz and

karger@karger.com
www.karger.com/cgr

Késsia L. Souza
Departamento de Biologia Animal, Universidade Federal de Viçosa
Avenida PH Rolfs
Viçosa, MG 36570-900 (Brazil)
kessialsouza@gmail.com

Table 1. Summarized data of the analyzed species

Species	Specimen, *n*	Sex	Sample locality	Voucher
Aplastodiscus weygoldti	2	Male	RPPN Miguel Feliciano Abdala, Caratinga (MG)	MZUFV 15935, UFMG-A 13166
	1	Female		UFMG-A 13167
Aplastodiscus cavicola	4	Male	Viçosa (MG)	MZUFV 15924, 15925, 15926, 15927
	1	Male	Cataguases (MG)	MZUFV 16762
Aplastodiscus sp. 4	7	Male	Alvorada de Minas (MG)	MZUFV 15928, 15929, 15930, 15931, 15932, 15933, 15934
Aplastodiscus sp. 6	2	Male	Pedra Dourada (MG)	MZUFV 14528, 14527

Peixoto, 1984, 1985; Garcia et al., 2001; Cruz et al., 2003; Faivovich et al., 2005; Berneck et al., 2016; Ferro et al., 2018; Bezerra et al., 2020; Frost, 2020].

The phylogenetic position of Hylidae [sensu Duellman et al., 2016] suggests that the most common diploid number 2n = 24 is a possible synapomorphy of the family [Faivovich et al., 2005; Catroli and Kasahara, 2009], with some divergencies, such as the presence of B chromosomes in the species *Bokermannohyla luctuosa* and *Boana albopunctata*. Among the Hylidae genera, *Aplastodiscus* stands out for having a high karyotypic diversity, 2n = 24, 2n = 22, 2n = 20, and 2n = 18, which allows the separation of species groups based on cytogenetic characters. This monophyly is also supported by other phylogenies [Faivovich et al., 2005; Duellman et al., 2016]. Several mechanisms have been proposed to explain the diploid number variation within *Aplastodiscus*, such as independent chromosomal rearrangements leading to chromosomal reductions [Gruber et al., 2012; Berneck et al., 2016].

Two species of the *A. perviridis* group (*A. perviridis* and *A. cochranae*) have 2n = 24 chromosomes, with mainly centromeric C-banding pattern. The nucleolus organizer regions (NORs) are located in the terminal region of the long arm of chromosome pair 12 in *A. cochranae*. In *A. perviridis*, NORs have been reported in chromosome pair 11 [Gruber et al., 2012] or 12 [Carvalho et al., 2009b].

All species of the *A. albofrenatus* group have 2n = 22 chromosomes, with the exception of *A. musicus*, recently reallocated to this species group, in which the karyotype is not known yet [Carvalho et al., 2009a; Gruber et al., 2012; Bezerra et al., 2020]. Heterochromatic blocks are centromeric in most chromosomes. NORs are always telomeric and restricted to chromosome pairs 1 and 6 in *A. albofrenatus*, chromosome pair 10 or 11 in *A. arildae*, chromosome pair 6 or 7 in *A. eugenioi*, and chromosome pairs 6 and 10 in *A. ehrhar*dti [Carvalho et al., 2009a; Gruber et al., 2012].

The group *A. albosignatus,* the least studied group, shows ambiguity in cytogenetic data: the species *A. albosignatus* [sensu Berneck et al., 2016] has 2n = 20 chromosomes, and *A. leucopygius* has 2n = 18 chromosomes. C-banding markings are predominantly centromeric in all chromosomes, and the NORs are located in the terminal region in chromosome pair 9 [Carvalho et al., 2009b; Gruber et al., 2012; Berneck et al., 2016]. Finally, species of the recently proposed group *A. sibilatus* have not been karyotyped yet [Berneck et al., 2016].

In this study, we characterized the karyotypes of 4 *Aplastodiscus* species for the first time, using classical and molecular cytogenetic techniques, such as Ag-NOR banding and FISH with an 18S rDNA probe. Finally, we propose an independent chromosome reduction within the *A. albosignatus* species group, to increase knowledge on the chromosomal evolution of the green clade.

Materials and Methods

In total, 17 adult specimens belonging to 4 species of *Aplastodiscus* were collected from various localities in Minas Gerais State, Brazil (Table 1). We sampled 1 species (*A. weygoldti*) from the *A. albofrenatus* group and 3 species (*A. cavicola*, *Aplastodiscus* sp. 4, and *Aplastodiscus* sp. 6, sensu Berneck et al. [2016]) from the *A. albosignatus* group. All vouchers were housed in the herpetological collection of the Museu de Zoologia João Moojen from the Universidade Federal de Viçosa (MZUFV) and the amphibian collection from the Universidade Federal de Minas Gerais (UFMG-A), both in Minas Gerais State, Brazil.

Table 2. Comparative chromosome morphology in the 4 species

Species		Chromosome pairs										
		1	2	3	4	5	6	7	8	9	10	11
Aplastodiscus cavicola	CR	1.14	1.49	1.80	2.18	2.32	2.15	2.35	2.55	1.34	–	–
	CT	m	m	sm	sm	sm	sm	sm	sm	m	–	–
Aplastodiscus sp. 4	CR	1.04	1.41	1.85	2.66	2.71	2.53	2.05	2.96	1.31	–	–
	CT	m	m	sm	sm	sm	sm	sm	sm	m	–	–
Aplastodiscus sp. 6	CR	1.17	2.13	2.13	2.18	1.82	3.04	2.38	3.06	1.27	–	–
	CT	m	sm	sm	sm	sm	st	sm	st	m	–	–
Aplastodiscus weygoldti	CR	1.05	1.09	1.63	2.59	3.04	2.94	2.26	1.29	1.48	1.27	1.34
	CT	m	m	m	sm	st	sm	sm	m	m	m	m

CR, centromeric ratio; CT, chromosome type; m, metacentric; sm, submetacentric; st, subtelocentric.

Mitotic chromosomes were obtained from gut epithelial cells of the specimens, according to Schmid [1978]. Each specimen was injected intraperitoneally with 0.1% colchicine solution (0.1 mL per 10 g of body weight) for 4 h before euthanasia (carried out with 5% lidocaine). The cells were incubated in hypotonic solution (0.9% sodium citrate) and fixed in Carnoy's solution (methanol:acetic acid, 3:1).

The slides with cell suspensions were stained using conventional protocols (5% Giemsa diluted in Sorensen buffer). The best metaphases were photographed in a digital Olympus BX53 light microscope with a DP73 Olympus camera. Chromosome pairing and measurements were performed using Image Pro Plus® (IPP Version 4.5) to determine the modal value (2n) and the fundamental number (FN) for each population. Homologs were paired and grouped according to the centromere position, in descending order of size. Finally, the chromosomes were classified according to their centromeric indices into metacentric (m), submetacentric (sm), and subtelocentric (st), following Green and Sessions [1991].

Active NORs in the preceding interphase were identified using silver nitrate precipitation (Ag-NORs) [Howell and Black, 1980]. The FISH technique was performed according to Pinkel et al. [1986], with modifications (denaturation with 70% formamide, at 75°C for 5 min). The 18S rDNA probe was obtained by PCR amplification, using the primers 18SF (5′-CCGAGGACCTCACTAAACCA) and 18SR (5′-CCGCTTTGGTGACTCTTGAT) and was labeled by nick translation with digoxigenin-11-dUTP. Signal detection and amplification were performed using isothiocyanate probe, fluorescein-conjugated anti-digoxigenin-rhodamine. FISH images were captured in a BX53 Olympus microscope with a XM10 camera.

Results

All species analyzed from the *A. albosignatus* group (*A. cavicola*, *Aplastodiscus* sp. 4, and *Aplastodiscus* sp. 6) had a complement of 18 chromosomes (Table 2). All chromosomes were biarmed, and their FN was 36. Chromosome pairs 1 to 7 were of larger size, with slight variation between chromosome pairs 2 to 7, chromosome pair 8 was medium-sized, and only one small-sized chromosome pair was present. Out of these 3 species, only *Aplastodiscus* sp. 6 had the chromosomal formula 4m + 10sm + 4st, showing 2 subtelocentric pairs (Fig. 1), whereas, *A. cavicola* and *Aplastodiscus* sp. 4 had 6m + 12sm chromosomes (Fig. 1). The representative of the *A. albofrenatus* group (*A. weygoldti*) showed 2n = 22 chromosomes and FN = 44, with chromosome pairs 1 to 5 of large size, chromosome pairs 6 and 7 of medium size, and chromosome pairs 8 to 11 of small size (Fig. 1). The chromosomal formula was 14m + 6sm + 2st. Heteromorphic sex chromosomes or supernumerary elements were not detected in any of these species.

The first pairs of all species are clearly metacentric. The smallest pairs are the last 4 pairs in *A. weygoldti* and the last one in the 3 species of the *A. albosignatus* group. The morphology and relative size of chromosome pairs 2 to 7 were similar in all species.

Secondary constrictions were observed in the long arms of chromosome pair 6 in *A. weygoldti*, and Ag-NORs were detected in the interstitial region of the short arm of the same chromosome pair. In the other 3 species (*A. cavicola*, *Aplastodiscus* sp. 4, and *Aplastodiscus* sp. 6), the Ag-NORs were located in the terminal region of the long arms of chromosome pair 9. FISH confirmed the position of the 18S rDNA sites in the terminal region of the long arms of both homologs of chromosome pair 9. In *A. weigoldti,* the 18S rDNA sites were observed in the interstitial region of the short arms on both homologs of chromosome pair 6.

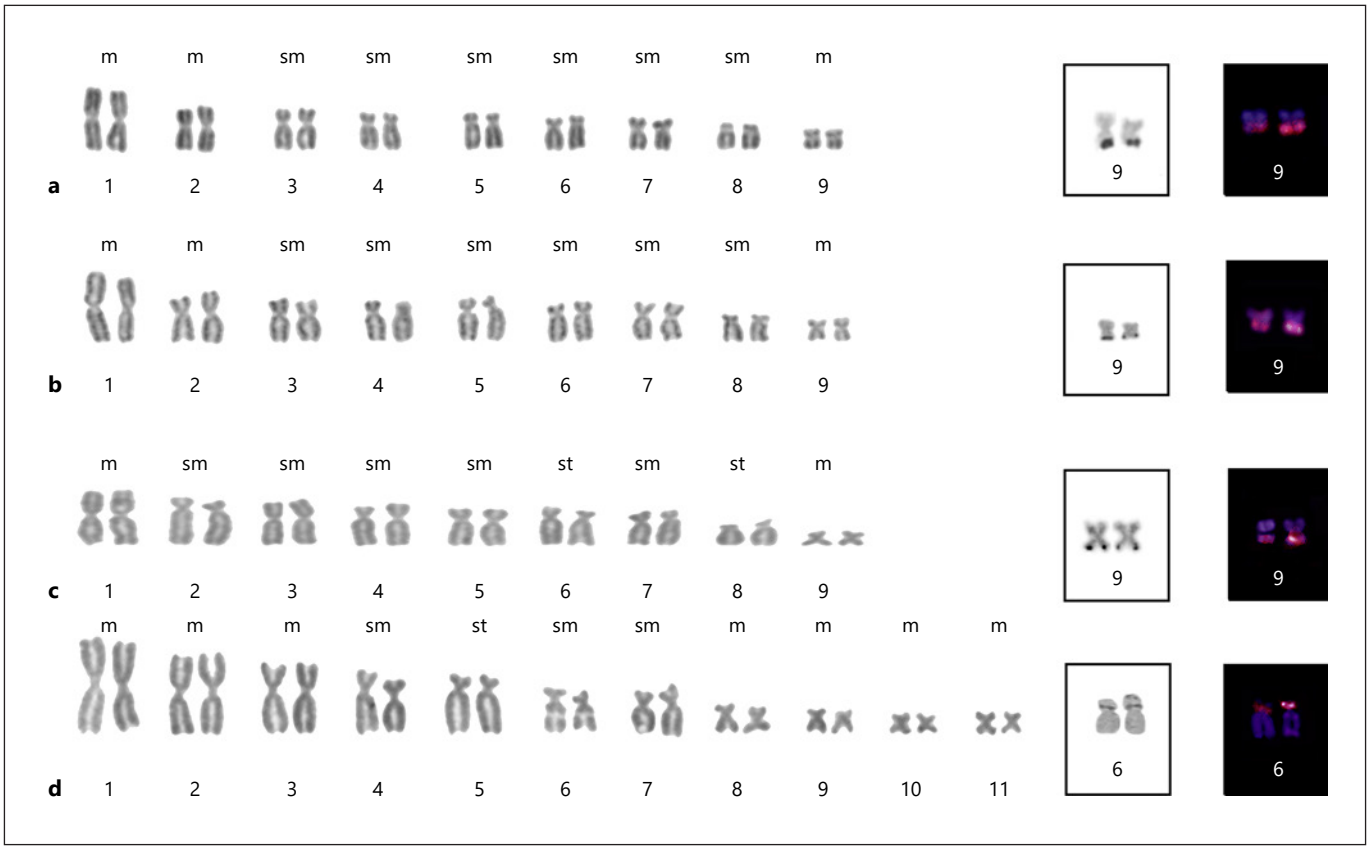

Fig. 1. Giemsa-stained karyotypes and NOR-bearing chromosomes after Ag-NOR staining and FISH with the 18S rDNA probe. **a** *Aplastodiscus cavicola*. **b** *Aplastodiscus* sp. 4. **c** *Aplastodiscus* sp. 6. **d** *Aplastodiscus weygoldti.*

Based on the optimization method proposed by Berneck et al. [2016], we added the diploid numbers of the species analyzed in the present study. It was possible to fill in some gaps – although there are still some critical species that lack chromosome information – and propose a new chromosomal rearrangement event that characterizes the *A. albosignatus* group, using the TNT and Adobe Illustrator software systems for reconstruction and editing of the tree (Fig. 2).

Discussion

The species analyzed in this study confirm the karyotypic variability of *Aplastodiscus* and the divergence from 2n = 24 chromosomes that characterizes hylids. Indeed, the observed diploid numbers were similar to those found in other species of Cophomantinae (i.e., *B. albopunctata* with 2n = 22 chromosomes and *Bokermannohyla ibitipoca* with 2n = 24 chromosomes) [Souza, 2019], which are also divergent from other species of Hylidae due to a high variation of the diploid number [Beçak, 1968; Bogart, 1973; Baldissera et al., 1993; Gruber et al., 2007; Ferro et al., 2012]. Considering the *Aplastodiscus* sp. 5 karyotype (see comments below), karyotype data are available for 13 species of the genus [sensu Berneck et al., 2016], but no karyotype has been reported for the *A. sibilatus* species group. Our analyses confirm the diploid numbers for the *A. albofrenatus* (2n = 22) and *A. albosignatus* (2n = 18) groups, as previously reported [Carvalho et al., 2009a, b; Gruber et al., 2012].

The species *A. albosignatus* was described considering Cubatão municipality, state of São Paulo (SP), as the type locality. Bogart [1973] found a diploid number of 2n = 20 chromosomes in this species based on individuals from Boracéia municipality (SP). In addition, Carvalho et al. [2009b], analyzing individuals from the populations of Piraquara municipality, state of Paraná (PR), and São Bento do Sul municipality, state of Santa Catarina (SC), reported diploid numbers of 2n = 20 chromosomes.

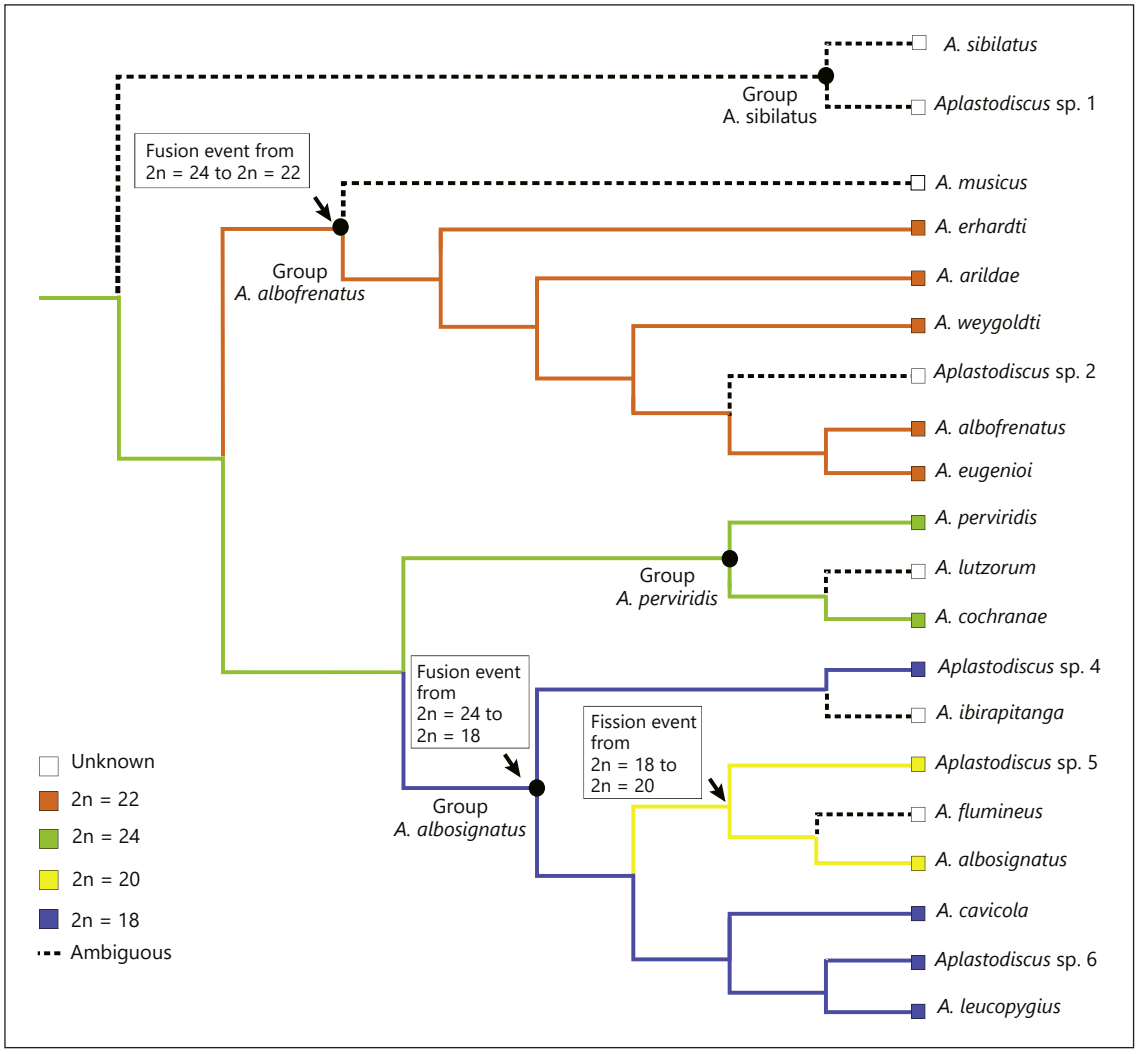

Fig. 2. Optimization of the diploid number in *Aplastodiscus* showing the chromosomal events as synapomorphies. Data used to reconstruct the phylogeny are from Bogart [1973], Carvalho et al. [2009a, b], Gruber et al. [2012], and Berneck et al. [2016].

However, Berneck et al. [2016], studying the same individuals as Carvalho et al. [2009b], classified the populations from São Bento do Sul (SC) as a new unnamed species (called *Aplastodiscus* sp. 5). In this way, the karyotype of *A. albosignatus* from São Bento do Sul belonged, indeed, to the new putative species *Aplastodiscus* sp. 5.

Thus, our results for the *A. albosignatus* group and the reidentification of the *Aplastodiscus* sp. 5 population, before acknowledged as *A. albosignatus* [Carvalho et al., 2009b], add new information that allows reformulating hypotheses of evolutionary chromosomal trends. Chromosomal fusion apparently occurred at the ancestral diploid number from 2n = 24 to 2n = 22 [Gruber et al., 2012], which is also observed in *A. weygoldti*, and this condition may also hold for *Aplastodiscus* sp. 2. These results further support the hypothesis of independent fusions occurring in the *A. albofrenatus* and *A. albosignatus* groups [Gruber et al., 2012; Berneck et al., 2016].

In addition, our data suggest that chromosomal fusion played a role in 2n = 24 to 2n = 18 karyotypes in the first proposed node of the *A. albosignatus* group. Chromosomal fission also occurred in the node of *Aplastodiscus* sp. 5 + *Aplastodiscus flumineus* + *A. albosignatus*, reversing the diploid number from 2n = 18 to 2n = 20 in *Aplastodiscus* sp. 5 and in *A. albosignatus*. This fact highlights a non-parsimonious evolutionary trend within the *A. albosignatus* species group. In addition, we propose that the character state observed in *A. flumineus* is a synapomor-

phy of the clade. High variation in the diploid number due to centric fusion or fission is frequently observed among the closest species, as found in one specimen of *A. leucopygius* that apparently presented a reciprocal translocation event [Guerra, 1988; Schmid et al., 2018]. Additionally, a reduced chromosome number increases the number of rearranged chromosomes for the establishment of specific species karyotypes, as observed in rodents [Fagundes and Yonenaga-Yassuda, 1998]. Thus, multiple and recurrent chromosomal rearrangements explain the diploid number variation of this genus. It will be interesting if prospective cytogenetic analyses of the *A. sibilatus* clade confirm the 2n = 24 plesiomorphic condition indicated for the genus.

Our results showed differences between the karyotype formulae described here and the karyotype formulae for other species of the group. Those rearrangements may play an important role in the biotic diversity, since postzygotic barriers depend on the fertility loss due to multiple chromosomal arrangements [Sites and Moritz, 1987; Guerra, 1988; Faria and Navarro, 2010]. *A. cavicola* and *Aplastodiscus.* sp. 4 exhibit karyotypic formulae similar to those of *A. albosignatus* and *A. leucopygius,* and this character condition may be a synapomorphy for these species.

The increased number of small chromosome pairs in species of the *A. albofrenatus* group, similar to *A. weygoldti* and *A. eugenioi,* when compared to species of *A. albosignatus* group, suggests that the events of fission and chromosomal fusion involved the larger chromosome. Based on the observed patterns, the chromosomal evolution from an ancestor with 2n = 24 chromosomes, for the species analyzed here, suggests 2 pathways of chromosomal rearrangement events: chromosome pairs 6 and 7 in species with 2n = 18 chromosomes (*A. cavicola*, *Aplastodiscus* sp. 4, and *Aplastodiscus* sp. 6) derived from the merger of the small chromosome pairs 7, 8, 9, and 10. Likewise, the large chromosome pair 2 of *A. weygoldti* (2n = 22) would result from fusion of the small chromosome pair 12 and the large chromosome pair 3 [Gruber et al., 2012].

The positions of the Ag-NORs and FISH signals of the 18S rDNA probe in chromosome pair 6 in *A. weygoldti* are similar to other species in the group, but differ to the presence of multiple NORs, as observed in *A. albofrenatus* and *A. ehrhardti* [Carvalho et al., 2009b]. The group *A. albosignatus* presents simple NORs in the species *A. cavicola*, *Aplastodiscus* sp. 4, and *Aplastodiscus* sp. 6. Ag-NORs were localized in chromosome pair 9, as observed in *A. albosignatus* and *A. leucopygius* [Carvalho et al., 2009a; Gruber et al., 2012]. In addition, a similar condition is observed in the *Boana* species groups [Ferro et al., 2018], which indicates high levels of conservation of these regions in this hylid species group.

Our study indicates new events of chromosomal rearrangements, combined with phylogenetic analyses, and increases the knowledge about the cytogenetic evolution of the genus *Aplastodiscus*. The hypothesis stated for the chromosomal evolution of the *A. albosignatus* group represents a new insight into the evolutionary trend for Cophomantinae, probably due to reverse rearrangements. The karyotype diversity within the genus contrasts with the conserved condition of typical hylid karyotypes. However, congruent markers of NOR and 18S rDNA are stable and characterize well-defined species groups.

Acknowledgements

K.L.S. is grateful to Patricia Santos, Marina Cunha, and Clodoaldo Assis for the collection of some specimens.

Statement of Ethics

Proceedings were carried out according to the regulations of the ethics committee on the use of animals in research of the Universidade Federal of Viçosa and the current Brazilian laws (CONCEA 1153/95).

Conflict of Interest Statement

The authors have no conflicts of interest to disclose.

Funding Sources

The authors are thankful to the "Coordenação de Aperfeiçoamento de Pessoa de Nível Superior" (CAPES) for its financial support.

Author Contributions

K.L.S. and M.A.P. prepared the cell suspensions, conducted the classical cytogenetic analyses and wrote the initial draft of the manuscript. K.L.S. and C.A.V.B. performed the FISH technique. R.N.F. and J.A.D. coordinated the research and revised the manuscript. All authors participated in writing and editing the manuscript.

References

Baldissera FA, Oliveira PSL, Kasahara S. Cytogenetics of four Brazilian Hyla species (Amphibia-Anura) and description of a case with a supernumerary chromosome. Rev Bras Genét. 1993;16:335–45.

Beçak ML. Chromosomal Analysis of Eighteen Species of Anura. Caryologia. 1968;21(3):191–208.

Berneck BVM, Haddad CFB, Lyra ML, Cruz CAG, Faivovich J. The Green Clade grows: A phylogenetic analysis of Aplastodiscus (Anura; Hylidae). Mol Phylogenet Evol. 2016;97:213–23.

Bezerra AM, Passos LO, de Luna-Dias C, Quintanilha AS, de Carvalho-e-Silva SP. A Missing Piece of the Puzzle: Re-Encounter of Aplastodiscus musicus, its Call, and Phylogenetic Placement (Anura: Hylidae: Cophomantini). Herpetologica. 2020;76(1):74–8.

Bogart JP. Evolution of anuran karyotypes. In: Vial JL, editor. Evolutionary biology of the anurans. Columbia: University of Missouri Press; 1973. p. 337–49.

Carvalho KA, Garcia PC, Recco-Pimentel SM. NOR dispersion, telomeric sequence detection in centromeric regions and meiotic multivalent configurations in species of the Aplastodiscus albofrenatus group (Anura, Hylidae). Cytogenet Genome Res. 2009a;126(4):359–67.

Carvalho KA, Garcia PC, Recco-Pimentel SM. Cytogenetic comparison of tree frogs of the genus Aplastodiscus and the Hypsiboas faber group (Anura, Hylidae). Genet Mol Res. 2009b;8(4):1498–508.

Catroli GF, Kasahara S. Cytogenetic data on species of the family Hylidae (Amphibia: Anura): results and perspectives. Publ UEPG Ciencias Biol e da Saude. 2009;1:067–86.

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

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

Cruz CAG, Pimenta BVS, Silvano DL. Duas novas espécies pertencentes ao complexo de Hyla albosignata Lutz & Lutz, 1938, do leste do Brasil (Amphibia, Anura, Hylidae). Bol Museu Nacional. 2003;503:1–13.

Duellman WE, Marion AB, Hedges SB. Phylogenetics, classification, and biogeography of the treefrogs (Amphibia: Anura: Arboranae). Zootaxa. 2016;4104(1):1–109.

Fagundes V, Yonenaga-Yassuda Y. Evolutionary conservation of whole homeologous chromosome arms in the akodont rodents Bolomys and Akodon (Muridae, Sigmodontinae): maintenance of interstitial telomeric segments (ITBs) in recent event of centric fusion. Chromosome Res. 1998;6(8):643–8.

Faivovich J, Haddad CFB, Garcia PCA, Frost DR, Campbell JA, Wheeler WC. Systematic Review of the Frog Family Hylidae, With Special Reference To Hylinae: Phylogenetic Analysis and Taxonomic Revision. Bull Mus Nat Hist. 2005;294(1):1–240.

Faria R, Navarro A. Chromosomal speciation revisited: rearranging theory with pieces of evidence. Trends Ecol Evol (Amst). 2010;25(11):660–9.

Ferro JM, Marti DA, Bidau CJ, Suárez P, Nagamachi CY, Pieczarka JC, et al. B Chromosomes in the Tree Frog Hypsiboas albopunctatus (Anura: Hylidae). Herpetologica. 2012;68:482–90.

Ferro JM, Cardozo DE, Suárez P, Boeris JM, Blasco-Zúñiga A, Barbero G, et al . Chromosome evolution in Cophomantini (Amphibia, Anura, Hylinae). PLoS One. 2018;13(2):e0192861–29.

Frost DR: Amphibian Species of the World: an Online Reference. Version 6.1. American Museum of Natural History, New York, USA; 2020. https://amphibiansoftheworld.amnh.org/index.php

Garcia PCA, Caramaschi U, Kwet A. O status taxonômico de Hyla cochranae Mertens e recaracterização de Aplastodiscus A. Lutz (Anura, Hylidae). Rev Bras Zool. 2001;18:1197–218.

Green DM, Sessions SK. Nomenclature for chromosomes. Amphibian cytogenetics and evolution. San Diego: Academic Press; 1991. p. 223.

Gruber SL, Haddad CF, Kasahara S. 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. 2007;130(3):281–91.

Gruber SL, Zina J, Narimatsu H, Haddad CF, Kasahara S. Comparative karyotype analysis and chromosome evolution in the genus Aplastodiscus (Cophomantini, Hylinae, Hylidae). BMC Genet. 2012;13:28–9.

Guerra M: Introdução à citogenética geral. Rio de Janeiro: Editora Guanabara Koogan; 1988. p. 1–142.

Howell WM, Black DA. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia. 1980;36(8):1014–5.

Pinkel D, Straume T, Gray JW. Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proc Natl Acad Sci USA. 1986;83(9):2934–8.

Schmid M. Chromosome banding in Amphibia. I. Constitutive heterochromatin and nucleolus organizer regions in Bufo and Hyla. Chromosoma. 1978;66:361–88.

Schmid M, Steinlein C, Haaf T, Feichtinger W, Guttenbach M, Bogart JP, et al. The Arboranan Frogs: Evolution, Biology, and Cytogenetics. Cytogenet Genome Res. 2018;155:1–247.

Sites JW, Moritz C. Chromosomal evolution and speciation revisited. Syst Zool. 1987;36(2):153–74.

Souza KL. Análise citogenética comparativa de três subfamílias de hilídeos (Amphibia: Anura) do estado de Minas Gerais [dissertation]. Universidade Federal de Viçosa; 2019.