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Source: Primate Conservation, 2010(25) : 21-26

Published By: Conservation International

URL: <https://doi.org/10.1896/052.025.0106>

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An *Aotus* Karyotype from Extreme Eastern Colombia

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Abstract: The paper ‘*Aotus* diversity and the species problem’ (Defler and Bueno 2007, *Primate Conserv.* 22: 55–77) reviewed the distribution of the *Aotus* karyotypes in Panama and Colombia. It included a discussion of a night monkey from Maipures, Vichada, Colombia, that we captured live and karyotyped for a project at the Universidad de Los Andes, Bogotá, Colombia, in 1983. In 1984, in an unpublished manuscript, we hypothesized that this specimen was a natural hybrid of *Aotus brumbacki*. Our identification was based on the karyotype G-band patterns, capture location, fur color, and a skin and skull preserved at the Instituto de Desarrollo de Los Recursos Naturales y Renovables (INDERENA), Bogotá, now at the Instituto de Alejandro von Humboldt. Defler and Bueno (2007) had interpreted the karyotype by assuming that all 50 chromosomes were paired. However, the authors had used data from an indistinct photograph of a karyotype without G-bands. This prompted us to review our original data using contemporary digital techniques to re-examine the G-band pattern origins of the chromosomes and further define the karyotype. We used precise chromosome G-band measurements and digital arm-ratio analyses to provide convincing evidence that the specimen is in fact a hybrid of *Aotus brumbacki*.

Key Words: Colombian primate, *Aotus brumbacki*, hybrid, G-banding karyotype, digital chromosome measurements

Introduction

In the early 1980s, we discussed Colombian *Aotus* diversity with the mammalogist Philip Hershkovitz of the Chicago Field Museum of Natural History, specifically concerning his 1983 revision of the genus (Hershkovitz 1983). Since one of us (TRD) was working close to the Río Orinoco, in Tuparro National Park, Colombia, Hershkovitz encouraged us to trap some night monkeys there, in order to confirm or refute the supposition that *Aotus trivirgatus* existed west of the Orinoco. *Aotus trivirgatus* was still unknown karyologically, and we hoped to provide geo-referenced Colombian specimens for this purpose. The precise provenance of most karyotyped specimens was unknown, creating problems for interpretation of karyotype origins and distributions. At that time the only geo-referenced collection site for a night monkey in Colombia was the holotype for *Aotus hershkovitzi* Ramirez-Cerquera, 1983, from Boyacá, on the eastern flanks of the Cordillera Oriental (see Defler *et al.* 2001). That collection site helped prove that the taxon was in fact a synonym of *Aotus lemurinus*. *Aotus lemurinus* had previously been associated with

karyotypes from Panama. The Panamanian karyotypes were subsequently interpreted to represent a distinct species, *Aotus zonalis* Goldman, 1914 (Hershkovitz 1983; Groves 2001; Defler 2004).

Following Hershkovitz’ request, we trapped a female night monkey at Maipures, Vichada, on the west (Colombian) bank of the Río Orinoco in 1983. Under the subtitle “*Aotus brumbacki* and the Maipures specimen”, Defler and Bueno (2007) discussed the identity of this specimen based on a poorly reproduced copy of a photomicrograph of its chromosomes, resulting from the work of M. V. Monsalve, R. Oliveira and T. R. Defler at the Universidad de Los Andes in 1984. In analyzing the image, Defler and Bueno (2007) indicated four pairs of metacentric, nine pairs of submetacentric (two of which were poorly resolved, but believed to be submetacentric), and eleven pairs of acrocentric chromosomes, along with one pair of sex chromosomes. They identified the specimen as *Aotus brumbacki* Hershkovitz, 1983 (p.217), based on the *A. trivirgatus trivirgatus* karyotype of Yunis *et al.* (1977), which Hershkovitz (1983) had synonymized with *A. brumbacki*. Defler and Bueno (2007) pointed

out, however, that “none of three previously published descriptions of chromosomal morphology for *A. brumbacki* (Brumback 1974; Yunis *et al.* 1977; Torres *et al.* 1998) agreed completely in the characteristics of the $2n = 50$ chromosome types, and showed considerable variation in the identification of numbers of metacentric, submetacentric and acrocentric chromosomes.” (p.58). They concluded that *A. brumbacki* should be subjected to further studies of its chromosome morphology. *Aotus brumbacki* is characterized by five pairs of “median-submedian” metacentric, seven pairs of subterminal and twelve pairs of terminal autosomal chromosomes. The sex-chromosome pair consists of a median X-chromosome and a small terminal Y-chromosome (Brumback 1974). The Deffler and Bueno (2007) interpretation of the Maipures specimen lacked cytogenetic analyses of chromosome arm lengths.

The genus *Aotus* shows considerable variation in the number of chromosomes. De Boer (1974) described diploid numbers of 49 and 51 in *A. trivirgatus* ssp., while Yunis *et al.* (1977) found diploid numbers of 50 and 54 in *Aotus trivirgatus*. For *A. trivirgatus griseimembra*, Yunis *et al.* (1977) found karyotypes with diploid numbers of 50, 52 and 54. Brumback *et al.* (1971) and De Boer (1971, 1972, 1974) described karyotypes with diploid numbers of 52, 53 and 54 (resulting from Robertsonian polymorphisms) also in *A. trivirgatus griseimembra*.

A study of 35 Colombian *Aotus* by Torres *et al.* (1998) showed karyotypes with diploid numbers from 46 to 58. The distribution of the *Aotus* in this study covered the area from 8°40'0"N to 4°12'55"S and 75°40'52"W to 69°56'26"W. Our Maipures *Aotus* from the west bank of the Río Orinoco has a karyotype with a diploid number of $2n = 50$, as do a number of specimens reported by Torres *et al.* (1998) from the Department of Meta nearby.

Here we expand the karyotype analyses of the Maipures specimen by: 1) classifying the chromosomes based on visual observation of G-band patterns; 2) digitally calculating chromosome arm ratios and total chromosome lengths; and 3) digitally calculating the distribution of banding patterns to match chromosomes. We explain the analyses of the G-band pattern of the karyotypes that support our earlier identification of the *Aotus* specimen.

Methods

In 1983, we trapped two living night monkeys from a group in a tree hollow on the west bank of the Río Orinoco, at Maipures, Department of Vichada (5°12'51.69"N, 67°50'03.44"W). We determined the karyotype of a female (IAvH4105) in the Laboratory of Human Genetics of the Universidad de Los Andes, Bogotá, Colombia. We delivered the two specimens (IAvH3888 and IAvH4105) to the Instituto de Desarrollo de Los Recursos Naturales y Renovables (INDERENA), and both are now part of the permanent collection at the Alexander von Humboldt Institute, Claustro de San Agustín, Villa de Leiva, Boyacá, Colombia.

We cultured 5 ml of the peripheral blood of the *Aotus* specimen IAvH4105 for 72 hours at 37°C in 5 ml of RPMI 1603 medium (Grand Island Biological) supplemented with 20% fetal calf serum and 0.2 ml of phytohemagglutinin M (Difco), according to a modification of the method by Moorhead *et al.* (1960). We obtained prometaphase chromosomes using the amethopterin cell synchronization technique developed by Yunis (1976). The cells were spread on a slide, and the slide was then air-dried and stained with Giemsa. Our study used the G-banding technique described by Sumner *et al.* (1973). We karyotyped well-spread prometaphase chromosomes obtained by screening fifty mitoses with chromosome complements of $2n = 50$.

We arranged the karyotypes according to the Miller *et al.* (1977) criteria to differentiate species of *Aotus*, arranging the G-band chromosomes with metacentrics first, then submetacentrics and acrocentrics last. We measured the length of short and long arms of our specimen using National Institutes of Health ImageJ (US National Institutes of Health) and Acrobat PDF software. We analysed each of the chromosomes in the karyotype photomicrographs using three different analyses: 1) ImageJ, 2) ImageJ with the option “invert,” and 3) ImageJ with the option “converting band density to peak data.” Using the results of these digital approaches, we re-organized the karyotypes in three groups according to the mean arm ratio values as follows: metacentric arm ratio, 1-1.9; submetacentric plus subtelocentric arm ratios, 2-3.9; acrocentric plus telocentric arm ratio, >4.0.

The reference karyotype of *Aotus brumbacki* was obtained from the photomicrograph in the journal article by Brumback (1974). We measured the arm ratio (length of the long arm divided by the length of the short arm) and total complement length for each chromosome using National Institutes of Health ImageJ (US National Institutes of Health 2009), and Acrobat PDF software. We analysed each chromosome of this karyotype with ImageJ and ImageJ with the option “invert”. The option “converting band density to peak data” in ImageJ was not used in the analyses because this karyotype did not have bands.

We expressed the length of each chromosome of our specimen and of *Aotus brumbacki* (Brumback 1974) as the percentage of the X-containing haploid complement length (%TCL) according to Torres *et al.* (1998). Results of these chromosome measurements obtained from our specimen and the *Aotus brumbacki* karyotype (Brumback 1974) are shown in Table 1.

Our research was approved by the *Comité de Investigaciones* of the Universidad de los Andes, Bogotá, Colombia. It complied with national requirements to leave biological samples with INDERENA, and it adhered to the American Society of Primatologists' principles for the ethical treatment of nonhuman primates.

Results

Our analyses of G-band chromosomes in the karyotypes of the Maipures specimen and the holotype were consistent with the existence of two different haploid complements with different numbers of metacentric and submetacentric chromosomes, but with identical numbers of acrocentrics and haploid complements, each consisting of 25 chromosomes. Each chromosome was classified as metacentric, submetacentric or acrocentric by measuring digital images according to the G-band distribution and arm length ratios. The peak data obtained with ImageJ allowed us to match five metacentric pairs, six submetacentric pairs, and nine acrocentric chromosome pairs as homologous. Some chromosomes were not homologous. We therefore divided the chromosomes into two groups, each one with a haploid complement of 25 chromosomes. Group I (Haploid A in Fig. 1) consisted of six metacentric (including one X-chromosome), seven submetacentric and twelve

acrocentric chromosomes. Group II (Haploid B in Fig. 1) consisted of seven metacentric (including one X-chromosome), six submetacentric and twelve acrocentric chromosomes. We show the G-banding karyotype of the *Aotus* IAvH4105 with 50 chromosomes (Fig. 1). The chromosome measurements of the *Aotus* karyotypes are expressed as percentages of the total chromosome length (TCL) and mean of the arm ratio (Table 1).

These findings are consistent with the existence of two different haploid complements with 25 chromosomes each. We confirmed the preliminary conclusions in our unpublished 1983–1989 *Aotus* project indicating that the specimen was an *Aotus brumbacki* hybrid. Using digital tools we identified chromosomes that did not match as pairs according to their G-band patterns and in their long- and short-arm ratios.

The accepted morphological description of the karyotype for *Aotus brumbacki* is that of Brumback (1974) of a male specimen, originally referred to as *Aotus azarae*

Table 1. Chromosome measurements of the Maipures specimen's two haploid complements ($n = 25$) and the diploid *Aotus brumbacki* complement ($2n = 50$).

Haploid Complement A					<i>Aotus brumbacki</i> ** Complement				Haploid Complement B				
	%TCL		Arm Ratio		%TCL		Arm Ratio			%TCL		Arm Ratio	
Chromosome	x	s	x	s	x	s	x	s	Chromosome	x	s	x	s
1 ^M	6.1	0.74	1.5	0.32	6.7	7.95	1.8	0.14	1 ^M	6.3	4.30	1.3	0.22
2 ^M	5.6	0.56	1.2	0.13	5.8	2.69	1.2	0.25	2 ^M	4.9	4.37	1.5	0.14
3 ^M	3.9	1.09	1.7	0.14	4.1	5.00	1.5	0.24	3 ^M	4.4	4.09	1.3	0.08
4 ^M	3.9	2.37	1.6	0.53	3.8	7.76	0.9	0.11	4 ^M	4.1	4.15	1.5	0.32
5 ^M	3.5	3.39	1.3	0.21	4.0	3.84	1.7	0.14	5 ^M	3.3	1.98	1.6	0.24
6 ^S	5.9	2.10	2.7	0.34	5.7	1.47	5.0	1.02	6 ^M	4.7	7.83	1.8	0.07
7 ^S	4.6	1.92	3.2	0.18	5.7	7.81	7.3	0.76	7 ^S	6.1	3.46	2.6	0.22
8 ^S	4.4	0.95	3.6	0.63	5.6	3.58	3.1	0.03	8 ^S	4.7	2.01	3.3	0.16
9 ^S	4.2	1.82	3.5	0.30	4.9	1.33	3.0	0.55	9 ^S	4.2	2.53	3.0	0.37
10 ^S	3.6	2.90	3.8	0.29	4.6	2.06	3.1	0.15	10 ^S	4.1	1.91	3.5	0.70
11 ^S	3.7	2.21	2.3	0.21	4.3	0.62	2.6	0.15	11 ^S	4.0	0.71	2.9	0.58
12 ^S	2.9	1.78	2.9	0.28	3.5	12.22	2.3	1.02	12 ^S	4.0	1.52	2.1	0.03
13 ^A	5.3	2.48	5.6	0.26	4.4	12.43	5.6	2.73	13 ^A	5.3	2.56	5.9	0.40
14 ^A	4.6	1.71	9.3	2.68	4.1	1.19	11.5	7.39	14 ^A	4.4	2.17	8.8	1.05
15 ^A	4.0	3.19	6.3	1.75	3.6	18.19	5.8	3.77	15 ^A	3.8	3.29	4.7	0.16
16 ^A	4.2	2.73	4.8	0.10	3.4	4.73	8.2	3.95	16 ^A	3.7	2.67	4.5	0.45
17 ^A	3.6	2.45	5.2	0.22	3.1	2.60	17.0	10.19	17 ^A	3.6	2.03	5.2	0.10
18 ^A	3.4	2.17	4.3	0.23	3.2	6.61	8.4	5.01	18 ^A	3.3	1.58	4.1	0.18
19 ^A	2.9	2.47	4.1	0.20	2.8	4.98	15.1	8.22	19 ^A	2.9	3.92	4.3	1.12
20 ^A	2.9	2.93	4.9	0.08	2.6	1.25	7.7	0.88	20 ^A	2.7	3.26	4.7	0.66
21 ^A	2.8	4.31	4.6	0.34	2.6	3.44	6.2	0.38	21 ^A	2.8	3.02	5.0	0.09
22 ^A	2.7	5.61	6.0	3.55	2.5	2.77	8.5	2.52	22 ^A	2.9	3.42	4.9	2.21
23 ^A	2.6	3.53	4.8	0.63	2.4	1.06	7.6	1.00	23 ^A	2.6	3.55	4.9	0.45
24 ^A	2.7	1.57	6.7	2.45	2.2	4.01	5.2	1.47	24 ^A	2.7	0.76	4.3	0.92
X ^M	5.3	3.69	1.3	0.30	4.4	0.00	1.7	0.00	X ^M	4.6	4.04	1.2	0.23

*%TCL (%Total Chromosome Length) = relative length; x = mean; s = standard deviation.

^M= Metacentric; ^S=Submetacentric; ^A=Acrocentric

**Measurements obtained from the *Aotus brumbacki* karyotype (Brumback 1974).

Humboldt, 1812, but corrected by Hershkovitz (1983) as representing the new species that he named *Aotus brumbacki*. This karyotype was shown in Figure 1 in Brumback (1974) and was published again by Hershkovitz (1983) (Figure 7: *Aotus brumbacki* [holotype] FMNH 123035 [head in alcohol]). We measured the chromosomes of this karyotype by using ImageJ and Acrobat PDF software and our results indicated that five pairs of chromosomes and the X-chromosome fell into the metacentric category with values less than 2, and seven fell into the submetacentric category. Although the short arms of the acrocentric chromosomes were difficult to measure, their values were higher than 4.0, falling into the category of acrocentric chromosomes. The mean ratio and relative lengths are shown in Table 1. These results confirmed that

our digital measurements are in agreement with the numerical values found in our Maipures *Aotus* specimen.

Our karyotype results of the Maipures specimen were validated by a methodology that included: 1) G-banding pattern examinations by four colleagues at the University of British Columbia (UBC) in addition to the first author; 2) separate analyses of digital images by two individuals working independently at UBC in addition to the first author; and 3) use of Image J and Acrobat PDF software to compare the results.

Discussion

Since some of the chromosomes in our specimen were difficult to classify either as metacentric, submetacentric

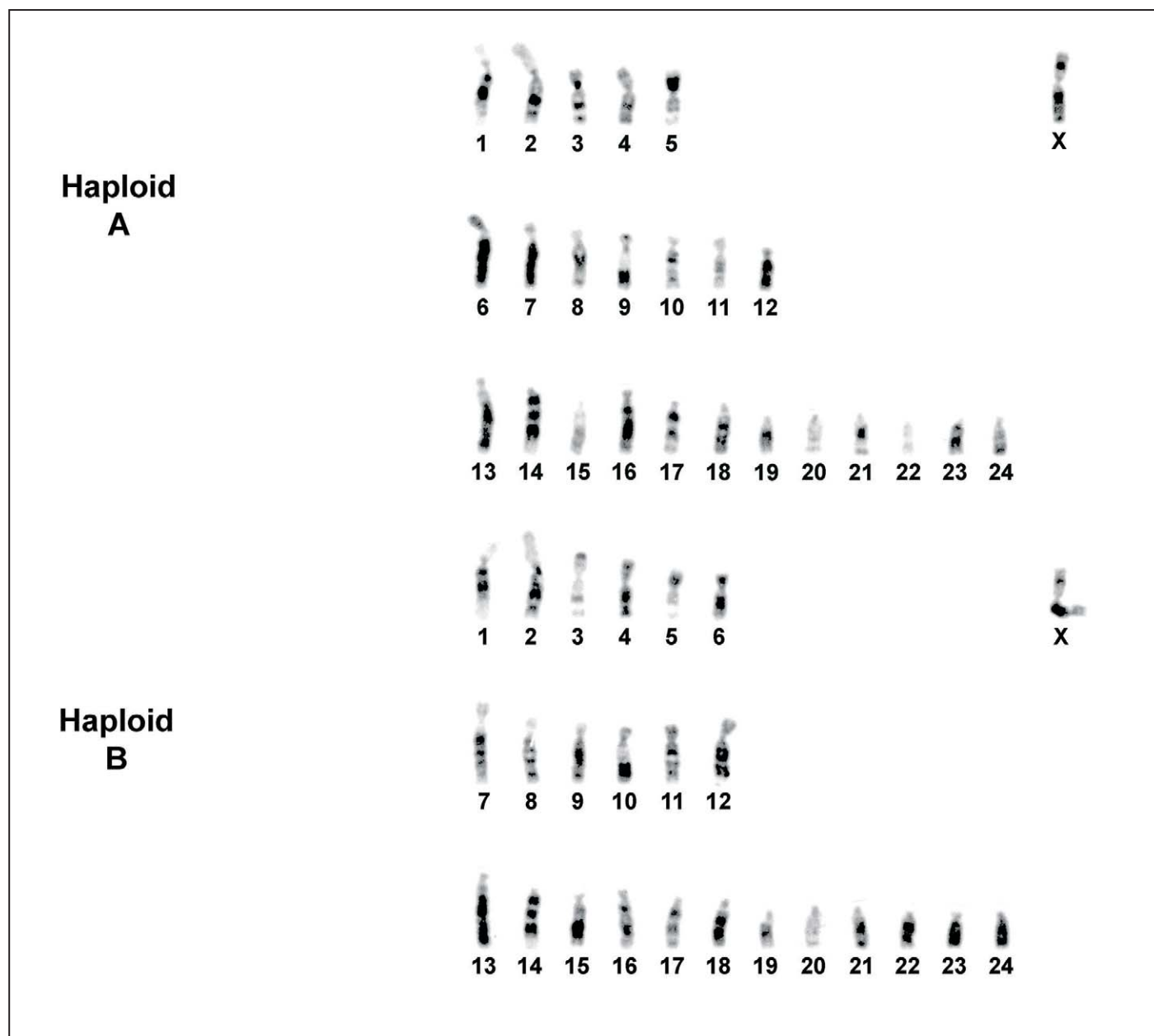


Figure 1. G-banding karyotype of the Maipures specimen with 50 chromosomes. The X chromosomes are at the right corner of Haploid A and Haploid B complements each one with 25 chromosomes. The chromosomes in both haploid complements are arranged in metacentric, submetacentric and acrocentric order.

or acrocentric, we used ImageJ and Acrobat PDF software to obtain digital measurements of long and short arms. This technique allowed us to classify each of the chromosomes of the karyotypes not only by visual observation of the G-band pattern but also more accurately based on quantitative values. We digitally plotted the bands of each chromosome and calculated the length of the short and long arms and bands. Their relative lengths helped us to determine if chromosomes were homologous. With these measurements we found that five chromosomes in the haploid complement “A” and six in the haploid complement “B”, and X-chromosomes of both complements, fell into the category of metacentric, with values less than 2.0. Seven chromosomes in the haploid complement “A” and six in the haploid complement “B” fell into the category of submetacentric, with values higher than 2.0. Finally, twelve chromosomes in haploid complements “A” and “B” fell into the category of acrocentric, with values higher than 4.0.

Our finding of odd numbers of metacentric and submetacentric chromosomes in this specimen, in addition to the presence of a diverse pattern of G-band chromosomes that are expected to be homologous, supports the hypothesis that this specimen is a hybrid. Our study sample may represent the karyotype of a natural hybrid resulting from the cross-breeding of *Aotus brumbacki* and another species. This second species should be searched for in eastern Colombia and probably at least partially overlaps with the distribution of *Aotus brumbacki*.

Our study suggests that one of the haploid complements may come from the population that Brumback (1974) described as having five pairs of “median-submedian” autosomal chromosomes, seven pairs of “subterminal” autosomal chromosomes and twelve pairs of “terminal” autosomal chromosomes in a male *Aotus brumbacki*.

We compared the Q-bands of the night monkey referred to as *Aotus trivirgatus trivirgatus* by Yunis *et al.* (1977) to the G-band patterns of our specimen. Depending on the particular staining technique the alternating light and dark or fluorescent and non-fluorescent bands in chromosomes can be seen under a microscope. A fluorescent band will be seen in a specific region of a chromosome using Q-band techniques while a dark band will be seen in the same region when using G-band techniques. None of the haploid complements of our specimen corresponded to those reported by Yunis *et al.* (1977) when we used ImageJ “invert” to digitally convert the G-band patterns of our specimen sample to Q-bands. Thus, the distribution of metacentric, submetacentric, and acrocentric chromosomes in our sample did not match the numerical distribution found in the *Aotus trivirgatus trivirgatus* reported by Yunis *et al.* (1977); a specimen considered by Hershkovitz (1983) to be synonymous with his *brumbacki*.

We did not find the metacentric, submetacentric nor acrocentric chromosome pair distribution that Torres *et al.* (1998) found in specimens with a diploid number of 50 chromosomes in Colombian *Aotus* specimens. That karyomorph study indicated five pairs of metacentric and submetacentric

and fourteen pairs of acrocentric chromosomes in the specimens from Meta (Colombia) and nine pairs of metacentric, three pairs of submetacentric and twelve pairs of acrocentric chromosomes in one specimen from Quindío, Colombia (later attributed to a new species, *Aotus jorgehernandezi* Defler and Bueno, 2007).

Our analyses of two karyotypes in our specimen do not support the interpretation offered by Defler and Bueno (2007). Digital chromosome G-band measurements as well as digital arm-ratio analysis was not done in our earlier work with the karyotype of the *Aotus* specimen. The novel methodological approach used in this study ensures a clear conclusion that our specimen is an *Aotus brumbacki* hybrid, and allows convincing classification of chromosomes otherwise difficult to classify using visual observation of banding patterns. This is of particular interest for hybrid specimens where chromosomes are difficult to pair as homologous.

Acknowledgments

We dedicate this paper to the memory of Philip Hershkovitz who encouraged our earlier work with intellectual and financial support. We are grateful to Roger Brumback at Creighton University for his review of the manuscript and insightful suggestions, Greg Doheney at the University of British Columbia, Canada, for his review and technical support, and Catherine Anderson, Adrian Burke, Syndy De Silva, Lisa McDonnell, Amanda Yeo, and Ian Wilson at the University of British Columbia for their technical assistance. We thank Roberto Olivares for assisting with the karyotype preparation, and the Universidad de Los Andes for providing financial support and a home for the project. We are especially grateful to Inderena and the National Parks System for permission to capture the animals for this work; we followed the national laws of compliance with animal care regulations.

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Received for publication: November 2010

Revised: 21 February 2011