Caribbean Journal of Science, Vol. 38, No. 3-4, 268–270

Karyotype of the Endemic Golden Frog *Atelopus zeteki* (Dunn) from Panama

CARLOS W. RAMOS[1], NILKA PIMENTEL[2], AND VÍCTOR MARTÍNEZ-CORTÉS[2] [1]*Departamento de Genética y Biología Molecular, Facultad de Ciencias Naturales Exactas y Tecnología, Universidad de Panamá, Estafeta Universitaria, República de Panamá. cramos@acon.up.ac.pa.* [2]*Departamento de Zoología, Facultad de Ciencias Naturales Exactas y Tecnología, Universidad de Panamá, Estafeta Universitaria, República de Panamá*

According to Savage (1972), the only species of *Atelopus* present in Costa Rica and western Panama are *A. varius*, *A. chiriquiensis*, and *A. senex*. However, other species such as *A. zeteki*, *A. certus*, and *A. glyphus*, reported by Savage as populations of *A. varius*, have been also recognized. Ibañez et al. (1995) reported *A. limosus* as a new species from Panamá. The taxonomy of most of these species is debatable partly because

TABLE 1. Nomenclature and centromeric ratio of the haploid set of *Atelopus zeteki* chromosomes. m = metacentric, sm = submetacentric.

Chromosome number	Chromosome type	Centromeric ratio (long arm/short arm)
1	m	1.25
2	m	1.33
3	m	1.61
4	sm	1.93
5	m	1.11
6	m	1.30
7	m	1.57
8	m	1.12
9	m	1.14
10	m	1.05
11	sm	2.02

they were described based on coloration and morphometric analyses.

Kim et al. (1975) considered that the presence of different toxins in *Atelopus* could be useful for biochemical taxonomy. Other taxonomic criteria, such as osteology, tadpole morphology, chromosome banding, and DNA polimorphism has been used infrequently. The only cytogenetic studies available are for *A. varius* (Duellman, 1967; Schmid, 1980) in which chromosome number and GC- and AT-rich chromosome regions are reported. We provide the first data for the karyotype of *Atelopus zeteki*, an endemic species Panamanian known as the golden frog.

On May 2000 we collected five *A. zeteki* (4 males, 1 female) from the pluvial premontane forest of Cerro Marta, Provincia de Coclé, Panama, (8° 41′ 18″ N, 80° 34′ 30″ W; 1000 m). The specimens were intraperitoneally injected with 0.05 % colchicine solution at 0.02 ml per g of body weight, killed after 24 h, and the bone marrow was collected in an Eppendorf tube. One specimen was deposited in the Vertebrates Museum, University of Panama (MVUP 1759). The hypotonic treatment and fixation steps lasted 45 min at room temperature. Chromosomes were stained adding 10 % Giemsa in phosphate buffer (pH 6.8) for 15 min and photographed with an FX-35DX camera and Kodak TMax 100 Professional film. The karyotype was constructed based on length and centromeric position.

Forty-three well-spread metaphase chromosomes were counted from the males (no results were obtained from the female). The modal number was 2N = 22 (14 cells), indicating that this is the diploid number for this species. The karyotype of *A. zeteki* (Fig. 1) was composed of 9 metacentric and 2 submetacentric chro-

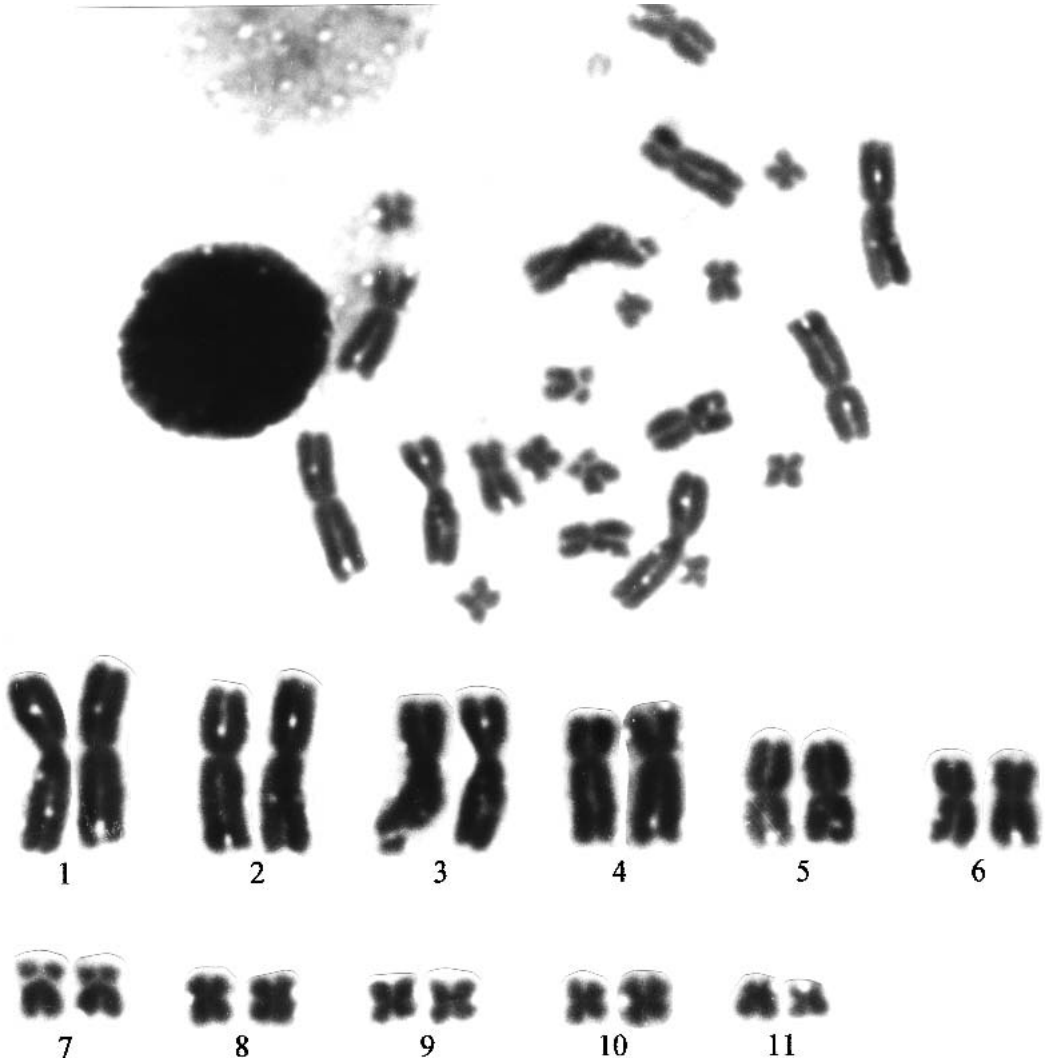

FIG. 1. Metaphase chromosomes and karyotype of male *Atelopus zeteki* (2N=22).

mosomes. To determine chromosome type we followed the centromeric position criteria of Levan et al. (1964). Chromosomes 1, 2, 3, 5, 6, 7, 8, 9 and 10 were classified as metacentric and chromosomes 4 and 11 as submetacentric, based on the ratio between the arms of each chromosome (Table 1).

According Kuramoto (1990), 86 of the 109 species of Bufonidae have 22 pairs of chromosomes. Although the chromosome number of *A. zeteki* is identical to that of *A. varius* (Duellman, 1967) and to that of the specimens examined by Barrera et al. (1984) now recognized as *A. ignescens* and *A. guanujo* (Coloma, 2002), some differences are observed in centromere position. In the specimens examined by Barrera et al. (1984), chromosomes 1, 2, 5, 6, 7, 8, 10 and 11 are metacentric and 3, 4 and 9 are submetacentric; while in *A. zeteki* chromosomes 3 and 9 are metacentric and 11 is submetacentric.

The fact that most bufonids exhibit no variation in chromosome numbers is consistent with the general opinion that chromosome evolution in frogs has been much slower than in other vertebrates (Bush et al., 1977). Subtle changes in chromosome morphology, such as reported here, have been observed in related genera of Bufonidae (Morescalchi and Gargiulo, 1968). The differences in centromere position between the chromosomes of *A. zeteki* and those of the specimens studied by Barrera et al. suggests that karyotype evolution in *Atelopus* has involved pericentric inversions.

Acknowledgments.—We are very thankful to Armando Rodríguez-Durán and Peter Meléndez for their comments on the manuscript. We also thank the Instituto Nacional de Recursos Naturales Renovables of the República de Panamá for providing collecting permits.

Literature Cited

Barrera, A. M., L. Lobato, and B. Araujo. 1984. Estudio cromosómico de dos especies de Anura (Hylidae-Bufonidae). Soc. Ecuador Biol., VIII Jord. Nac. Cienc. Biol. "Gustavo Orcés V." Vol. II: 419-427.

Bush, G. L., S. M. Case, A. C. Wilson, and J. L. Patton. 1977. Rapid speciation and chromosomal evolution in mammals. Proc. Natl. Acad. Sci. USA 74: 3942-3946.

Coloma, L. A. 2002. Two new species of Atelopus (Anura: Bufonidae) from Ecuador. Herpetologica. 58 (2):229-252.

Duellman, W. E. 1967. Additional studies of chromosome of anuran amphibiams. Syst. Zool. 16:38-43

Ibañez, R., C. Jaramillo, and F. Solís. 1995. Una especie nueva de *Atelopus* (Amphibia: Bufonidae) de Panamá. Carib. J. Sci. 31(1-2): 57-64.

Kim, H. Y., G. B. Brown, and H. Mosher. 1975. Tetrodotoxin: Ocurrence in Atelopid frogs of Costa Rica. Science, 185: 151-152.

Kuramoto, M. 1990. A list of chromosome numbers of Anuran Amphibians. Bull. Fukuoka Univ. Educ. 39: 83-127.

Levan, A., D. Fredga, and A. A. Sandberg. 1964. Nomenclature for centromeric positions on chromosomes. Hereditas 52: 201-220.

Morescalchi, A., and G. Gargiulo. 1968. Su alcune relazioni cariologiche del genere *Bufo* (Amphibia, Salientia). Rend. Acc. Sci. Fis. Mat., S. 4, 35:117-120.

Savage, J. M. 1972. The Harlequin frogs Atelopus, of Costa Rica and Western Panama. Herpetologica 28: 77-94.

Schmid, M. 1980. Chromosome banding in Amphibia. IV. Differentiation of GC- and AT-Rich Chromosome regions in Anura. Chromosoma 77:83-103.