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**Cytotaxonomic studies on Iberian and Moroccan *Pelobates* (*Anura*:
Pelobatidae)**

[With plate XVIII]

Cytotaksonomia rodzaju *Pelobates* (*Anura*: *Pelobatidae*) z Iberii i Maroka

Abstract. The karyotypes and C-banding patterns of *Pelobates cultripipes* and *P. varaldii* are described and compared. All the 13 pairs of biarmed chromosomes have the same nucleotypic characteristics in both species. Although the C-banding pattern is suggested to have some taxonomic value, in this extraordinarily conservative genus it does not represent an accurate systematic method.

I. INTRODUCTION

The genus *Pelobates* is represented in the Iberian Peninsula by *P. cultripipes* and in Morocco by *P. varaldii* (PASTEUR & BONS, 1959; MERTENS & WERMUTH, 1960). The controversy about the taxonomic position of *P. varaldii* either as a species (PASTEUR & BONS, 1959; ROČEK, 1981) or as subspecies (SAGE et al., 1982) has been solved by using electrophoretical and immunological analysis (BUSACK et al., 1985) which supports the specific status of *P. varaldii*.

In this study we examined the karyotypes and the heterochromatic regions of above mentioned spadefoot toads in order to increase the information pertaining to these two species.

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This paper is dedicated to Prof. M. MŁYNARSKI in recognition of his productive researcher life in the field of herpetology.

II. MATERIAL AND METHODS

The two species examined were obtained from the following localities: *P. cultripes* from Madrid and Cáceres (Spain), and *P. varaldii* from Kenitra (Morocco).

Chromosome preparations were made from epithelial gut and spermatogonial cells following the conventional techniques for orcein staining (HERRERO, 1982) and the SUMNER's method for heterochromatic differentiation of the chromosomes (SUMNER, 1972).

Several metaphases from each individual were photographed and relative length (length of each homologous chromosome pair with total length of the haploid set being 100) and arm ratio (long arm length/short arm length) were calculated with the measurements taken from enlarged photomicrographs. Chromosome pairs were numbered in the order of decreasing mean relative length and centromeric position was designated according to LEVAN et al. (1964). The karyotypes were compared with each other and the significance of species differences in chromosome pairs were tested.

Table I

Relative length and arm ratio of the chromosome pairs in the two pelobatid toads ($\bar{X} \pm \text{SE}$)

| Pair No. | <i>P. cultripes</i> | | <i>P. varaldii</i> | |
|----------|---------------------|-----------------|--------------------|-----------------|
| | relative length | arm ratio | relative length | arm ratio |
| 1 | 12.5 \pm 0.24 | 1.28 \pm 0.02 | 12.4 \pm 0.16 | 1.30 \pm 0.01 |
| 2 | 11.9 \pm 0.18 | 1.26 \pm 0.03 | 11.8 \pm 0.13 | 1.29 \pm 0.03 |
| 3 | 11.8 \pm 0.26 | 1.50 \pm 0.03 | 11.8 \pm 0.13 | 1.58 \pm 0.06 |
| 4 | 10.2 \pm 0.12 | 1.60 \pm 0.03 | 10.9 \pm 0.14 | 1.70 \pm 0.05 |
| 5 | 9.8 \pm 0.09 | 2.50 \pm 0.05 | 9.6 \pm 0.10 | 2.16 \pm 0.06 |
| 6 | 9.4 \pm 0.12 | 1.50 \pm 0.02 | 9.3 \pm 0.06 | 1.54 \pm 0.02 |
| 7 | 5.7 \pm 0.12 | 2.59 \pm 0.07 | 6.1 \pm 0.10 | 3.31 \pm 0.23 |
| 8 | 5.2 \pm 0.08 | 1.25 \pm 0.04 | 5.6 \pm 0.08 | 1.12 \pm 0.03 |
| 9 | 5.2 \pm 0.09 | 3.50 \pm 0.15 | 5.2 \pm 0.07 | 4.00 \pm 0.05 |
| 10 | 4.6 \pm 0.08 | 1.60 \pm 0.03 | 4.9 \pm 0.05 | 1.67 \pm 0.03 |
| 11 | 4.6 \pm 0.13 | 2.25 \pm 0.08 | 4.8 \pm 0.07 | 1.90 \pm 0.02 |
| 12 | 3.4 \pm 0.08 | 2.00 \pm 0.06 | 3.7 \pm 0.05 | 1.95 \pm 0.05 |
| 13 | 3.2 \pm 0.07 | 1.50 \pm 0.02 | 3.5 \pm 0.06 | 1.47 \pm 0.03 |

III. RESULTS AND DISCUSSION

The two species show 13 pairs of biarmed chromosomes consisting of six large and seven small pairs (pl. XVIII: 1).

All chromosomes, excepting the pairs 11 and 7, did not differ significantly both in relative length and arm ratio (Table I). The pairs 11 are different in their relative lengths and the pairs 7 can be distinguished by their arm ratio which could be related to the probable location of a NOR in the short arm

of this chromosome. In fact, size differences of the NORs homologue regions observed in some normal individuals in all anurans (SCHMID, 1982) change the short arm length and, as a consequence, the arm ratio. This variation has been described by SCHMID (1982) in two other species of the same genus (*P. fuscus* and *P. syriacus*).

The analysis of the C-banding pattern of both species does not supply any large differentiation. The heterochromatic distribution in their chromosomes exhibited equal characteristics according to the position and size of the bands (pl. XVIII: 2): large centromeric C-bands appear in the pairs 1, 2, 4, 9, and 12. Small centromeric C-bands in 3, 8, 10, 11 and 13 pairs. Pericentric C-bands in the short arms of 1 pair, and in the long arm of 8 pair. Telomeric C-bands in the long arms of 1, 2 and 11 pairs. Finally, the short arm of 7 pair is almost completely heterochromatic. This zone could be associated with the NOR-region like in other species of *Pelobates* (SCHMID, 1980).

The C-banding patterns obtained in these two species of Iberian and Moroccan toads are so identical that no consideration about their relationships as species or subspecies can be drawn. Their nucleotypic characteristics are as much conservative as the morphological ones (BUSACK et al., 1985). This is reinforced by the similarities observed between the C-banding pattern of *P. fuscus* analyzed by SCHMID (1982) and that of *P. cultripes* analyzed here (pl. XVIII: 2), which after other data are clearly phylogenetically separated (ROČEK, 1981).

In conclusion, though in general the chromosome C-banding patterns seem useful in differentiating taxonomically different forms, its use for members of the genus *Pelobates* is largely limited due to the extreme conservation of the group in this respect. It might be useful perhaps to adopt further more effective differential staining methods such as a BrdU-replication banding (SCHEMPF & SCHMID, 1981) to assess karyotypic evolution of these toads.

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STRESZCZENIE

W pracy opisano i porównano kariotypy i wzory prążków C u *Pelobates cultripes* i *P. varaldii*. Wszystkie pary chromosomów wykazują te same cechy u obu gatunków. Pomimo że zazwyczaj wzorowi prążków C przypisuje się duże znaczenie w badaniach taksonomicznych, nie znajduje to zastosowania u wyjątkowo konserwatywnego rodzaju, jakim jest *Pelobates*.

RESUMEN

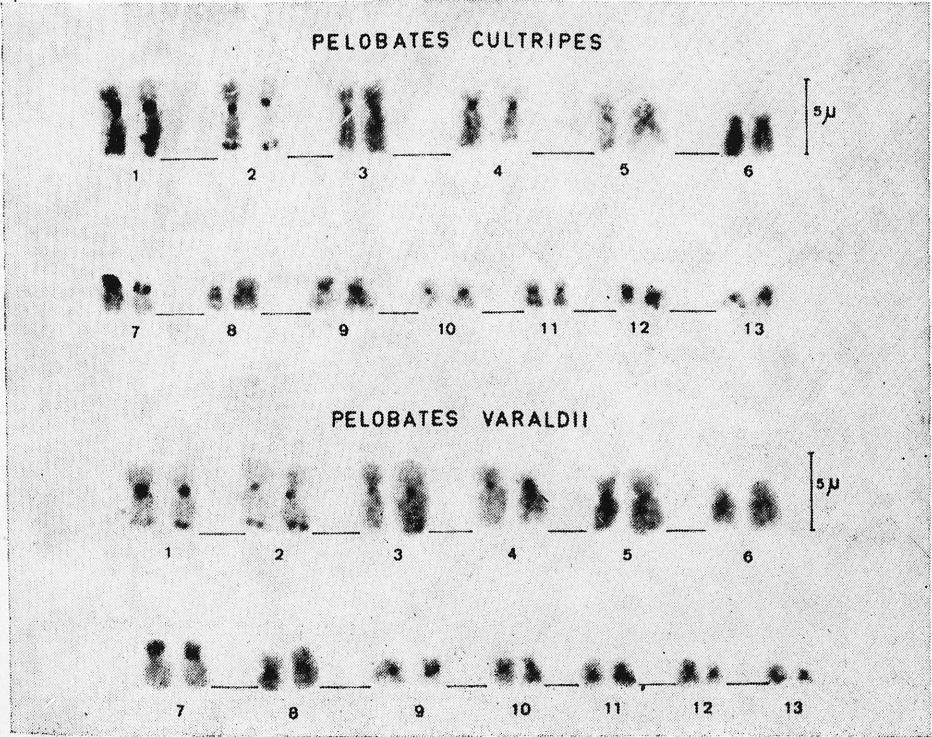
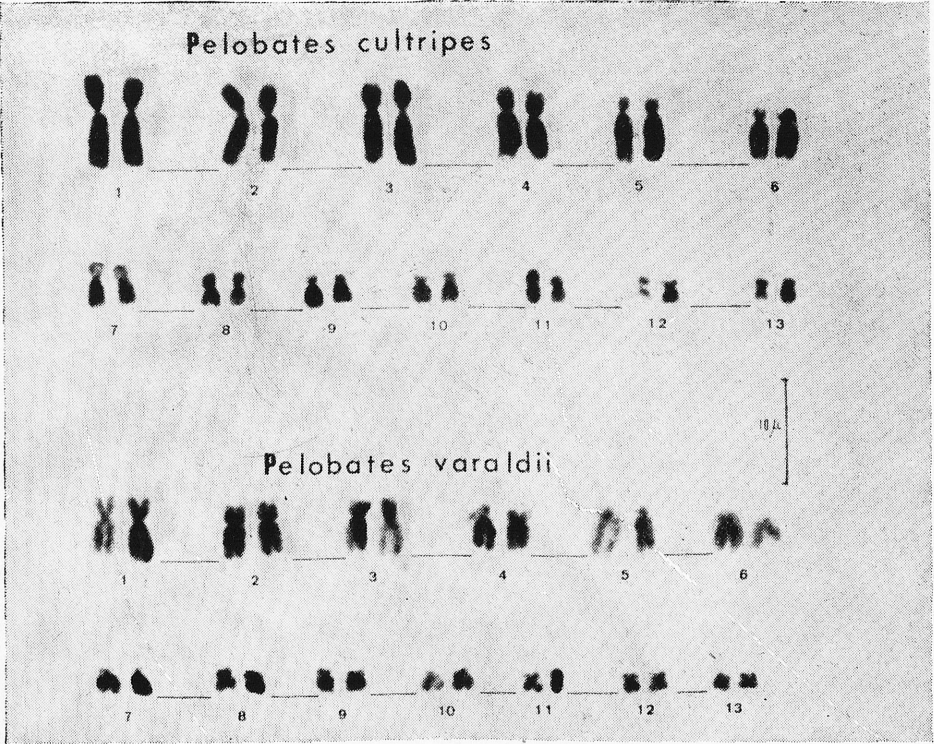
Se describen y comparan los cariotipos y patrones de C-bandeado de *Pelobates cultripes* y *P. varaldii*. Las características son las mismas en ambas especies. Aunque el patrón de bandas-C ha tenido una utilización taxonómica válida, en este género extraordinariamente conservativo, no representa un método sistemático seguro.

Edited by Dr. Z. Szyndlar

Plate XVIII

- 1 — Karyotypes of *P. cultripes* and *P. varaldii*
 2 — C-banded karyotypes of *P. cultripes* and *P. varaldii*

1



2