

C-BANDING KARYOTYPES AND LOCATION OF NOR'S IN THE TOAD SPECIES *BUFO BUFO* AND *BUFO VIRIDIS* FROM YUGOSLAVIA

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The karyotypes, C-banding patterns and positions of nucleolus organizer regions (NORs) of two European species of toads, *Bufo bufo* and *Bufo viridis* from seven localities in Yugoslavia were investigated. These karyotypes and chromosomal markers were compared with those in other species and subspecies of the *Bufo bufo* complex. The results suggest that C-banded patterns are characters specific for those species. A marker band on chromosome 5 in the karyotype of *B. bufo bufo* is shown.

Key words: *Bufo bufo*, *Bufo viridis*, constitutive heterochromatin, NORs

INTRODUCTION

Karyotypic conservatism characterizes many systematic groups of Amphibia (MORESCALCHI, 1973; WILSON *et al.*, 1974). The karyotypes of many amphibian species consist of meta- and submetacentric elements that can roughly be divided into "large" and "small" groups. Eighty three percent of toad species have a complement of $2n=22-26$ chromosomes (KURAMOTO, 1990). The considerable

differences in distribution of constitutive heterochromatin (C-bands) between some species (SCHMID, 1978; MATSUI *et al.*, 1985; GREEN and SESSIONS, 1991) question the notion of conservatism based on chromosome number and morphology. In spite of accumulating data on differences in C-band patterns between species there is little information about interpopulation variability of C-banding patterns in Amphibia (RAGGHIANI *et al.*, 1980; KING, 1980; GREEN, 1985; MIURA, 1995a).

The *Bufo bufo* complex includes many subspecies. Two subspecies inhabit the Balkan Peninsula. The described differences in karyotypes and C-banding patterns between *B. bufo* subspecies revealed intraspecific chromosomal variations (OBARA *et al.*, 1975; MATSUI, 1980; BIRSTEIN and MAZIN, 1982).

The taxonomy of green toads (*Bufo viridis* group) still lacks final clarification. Following a suggestion of ROTH (1996) real distinguishing is possible only between diploid and tetraploid toads (STOCK, 1998; CASTELLANO *et al.*, 1998).

The subject of the present study was cytogenetical characterization of two species that inhabit the Northern Balkans, namely the common toad *B. bufo* and the green toad *B. viridis*.

MATERIALS AND METHODS

A total of 17 specimens of toads from 7 different localities in Yugoslavia were cytogenetically analyzed (Table 1).

Table 1. - Collection localities and number of specimens examined

Species	Locality	males	females	total
<i>Bufo bufo</i>	Deliblatska peščara	-	1	1
	Lešnica	-	1	1
	Petnica	2	2	4
	Pašina ravan	-	1	1
	Tara (Mitrovac)	1	2	3
	Zlowska klisura	1	-	1
	Prokletije (Miliševački potok)	-	1	1
<i>Bufo viridis</i>	Petnica	2	3	5

The animals were treated with 0.3% colchicine solution 18-24 hours before chromosome preparation. C-bands were prepared according to the method of SCHMID (1978), with the modification described previously by SPASIĆ *et al.* (1996). The nucleolus organizer regions (NORs) were stained according to the procedure described by HOWELL and BLACK (1980). Constitutive heterochromatin was analysed on 20 selected metaphase figures. Measurements and comparisons of photometric profiles of chromosomes were done using a cooled SBIG ST7 CCD camera and the ANALYST computer program (MALKOV *et al.*, 1995).

RESULTS

The examined *B. bufo* and *B. viridis* specimens had symmetrical karyotypes that consisted of $2n = 22$ chromosomes (Fig. 1). Chromosomal pairs 1-6 were large and represented 80% of the genome, whereas small chromosomes 7-11 represented only 20 % of the genome.

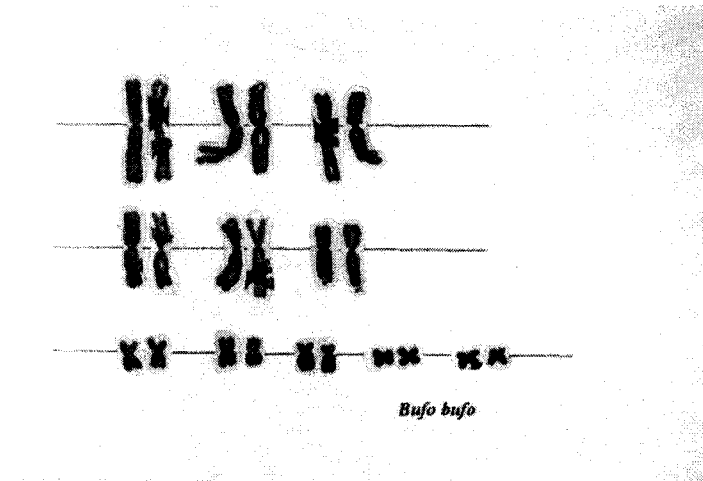


Fig. 1. Karyotype of *Bufo bufo*, conventional staining

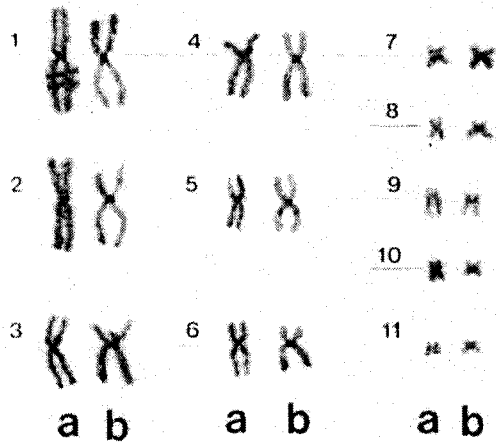


Fig. 2. C-banded chromosomes of A: *Bufo viridis*, B.: *Bufo bufo*

The karyotypes of the examined species showed differences in the amount and position of constitutive heterochromatin (Fig. 2). In both species, constitutive

heterochromatin was found only in the pericentromeric region of the small chromosome pairs, while the large chromosomes possessed constitutive heterochromatin in the centromere, as well as in the arms. The chromosome complement of *B. bufo* had more distinct C-positive heterochromatin in the centromeric regions than *B. viridis*. Comparative analysis of the distribution of constitutive heterochromatin provided information for a cytogenetic determination of the species (Fig. 3). The karyotype of *B. bufo* was characterized by C-bands at 5q (intercalary), 6q (telomeric), 2p (pericentromeric) and 7q (intercalary).

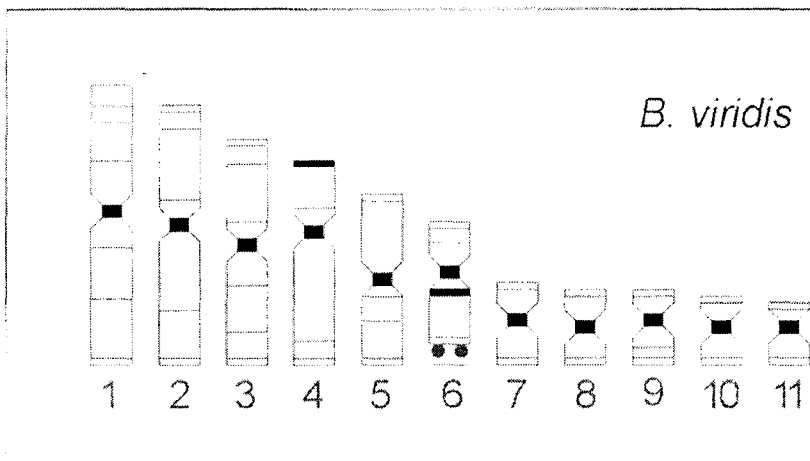
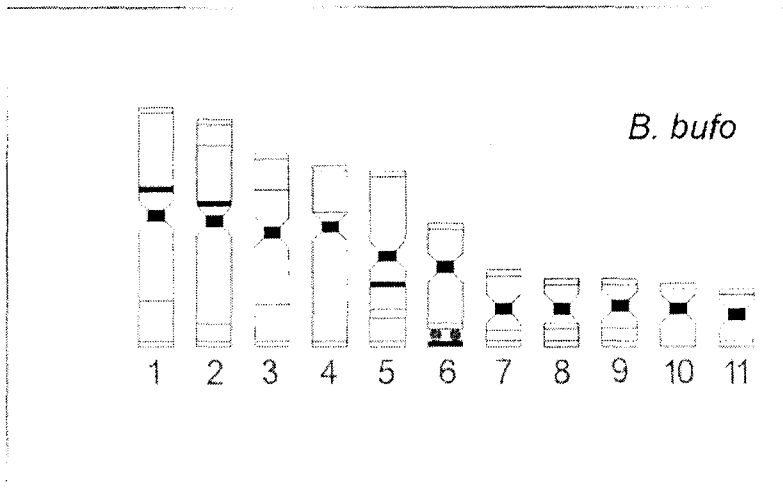


Fig. 3. Idiograms of C - banded karyotypes of the examined species

B. viridis had very characteristic chromosome pairs Nos. 1 and 3 with many C positive regions. Clear C-bands in the karyotype of *B. viridis* were located in the telomere on the short arm of the 4th chromosomal pair and in the pericentromeric region on the long arm of the 6th chromosomal pair. The most important difference in the distribution of constitutive heterochromatin was a very strong band in the intercalar position on the long arm of the 5th chromosome pair in *B. bufo*. Comparisons of photometric profiles of the 5th chromosome confirmed that (Fig. 5). This band was absent in the chromosome complement of *B. viridis*. Chromosome pair No. 5 also displayed a significant difference in relative chromosomal lengths, as well as in centrometic index.

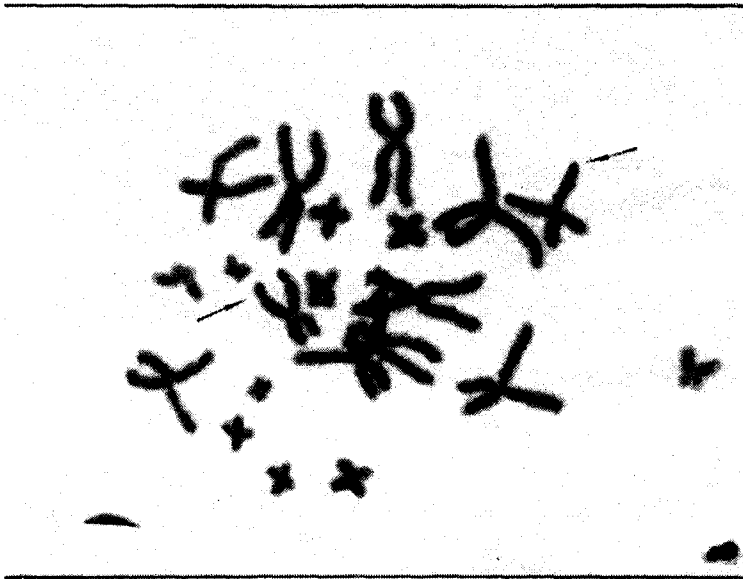


Fig. 4. Ag-NOR stained metaphase from *Bufo bufo*: Arrows show Ag-stained NORs of chromosome No.6

Interpopulation chromosome polymorphism, concerning general chromosome morphology and heterochromatin distribution, was not found in either species. Both species had the same position of NORs and corresponding secondary constrictions in the subtelomeric region on the long arms of the 6th chromosome pair (Fig. 3). In contrast to many populations where tetraploids of *B. viridis* were found, only diploid toads were found in the examined localities.

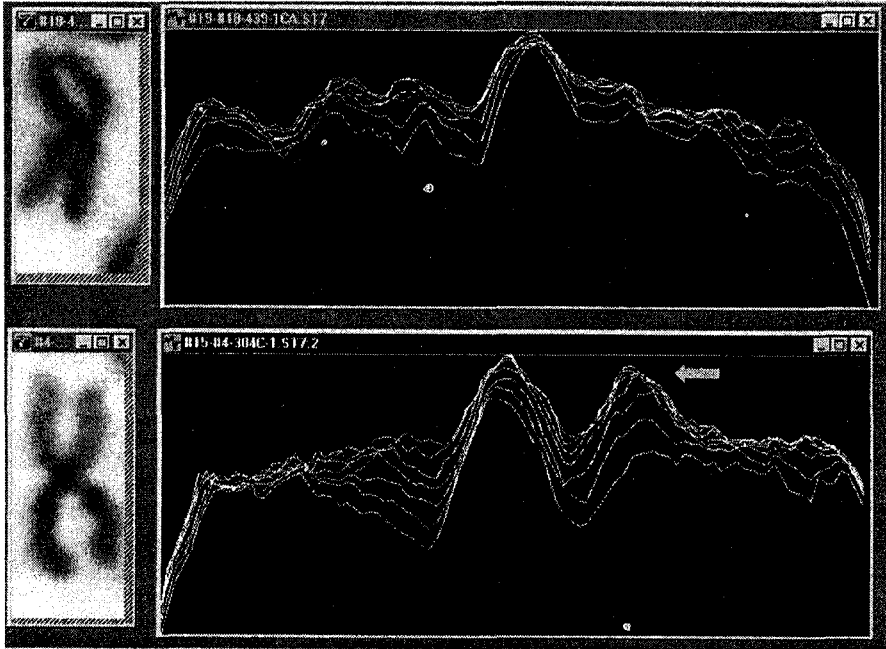


Fig. 5. Chromosome No. 5 selected for analysis and photometric profiles of chromosome No 5 of *Bufo viridis* (up) and *B. bufo* (down). Arrow shows marker band

DISCUSSION

Compared to other families of Anura, species of the family Bufonidae have been poorly karyologically and cytotaxonomically investigated. Much of the available data refers to Asian species and subspecies (OBARA *et al.*, 1975; MATSUI, 1980; MIURA, 1995a; 1995b; CASTELLANO *et al.*, 1998). The karyotype characteristics of European toads from the genus *Bufo* were described in considerable detail by SCHMID (1978). BIRSTEIN and MAZIN (1982) studied chromosome polymorphism of *Bufo bufo*. KING (1991) summarized that in the genus *Bufo* all species have dark paracentromeric C-bands, gray telomeric bands, C-bands associated with NORs, and that many chromosomes have interstitial bands in varying positions throughout the genome

Comparative chromosome analysis of species and subspecies of the genus *Bufo* revealed a conservative karyotype model. All of the chromosomes are either meta- or submetacentric, and C-positive heterochromatin is mostly found in centromeric and pericentromeric regions of chromosomes. All forms of European toads of the genus *Bufo* have NORs in the telomeric region in the 6th chromosome pair.

In spite of the conservatism of overall karyotype morphology, the distribution of constitutive heterochromatin varied considerably between the examined

species. *B. bufo* had more distinct C-positive heterochromatin in the centromeric regions than *B. viridis*. In the karyotype of *B. viridis* the chromosomes had many weak C-positive regions along the chromosome arms. In addition, *B. viridis* is the only European toad with a brightly fluorescing heterochromatin (SCHMID, 1978).

Examined specimens of *B. bufo* showed a very clear C-band on the long arm of the 5th chromosome pair (Fig. 2). The same position of the C-positive region was reported previously by SCHMID (1978) and BIRSTEIN and MAZIN (1982), who examined specimens from Germany and some localities near Moscow. MATSUI *et al.* (1985) described a 5p pc band in the karyotype of *B. bufo verrucosissimus*. The same 5p pc band was also observed in the karyotype of *B. japonicus* (MIURA, 1995a). According to observations reported by other authors and data presented here, we suggest that karyotype characteristics of the examined species based on the constitutive heterochromatin distribution are more regional - specific, than sub-species-specific.

While comparing the karyotypes of the *Bufo* species from different regions in Europe and Asia, we observed that 1 p pc and 6q tel C - positive regions are stable within the genus in spite of the observed differences in genome size (OLMO, 1973; BALDARI and AMALDI, 1976; BIRSTEIN and MAZIN, 1982).

Since subspecies of the *Bufo bufo* complex, as well as the *Bufo viridis* group, are morphometrically closely related, further cytogenetic, biochemical and bioacoustic analyses will be useful in identifying and characterizing the different forms in these complex groups of toads.

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**RASPORED C-TRAKA I LOKACIJA NUKLEOLUSNIH
ORGANIZATORA KOD DVE VRSTE ŽABA, *BUFO BUFO* I *BUFO
VIRIDIS* IZ JUGOSLAVIJE**

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Izvod

Ispitivani su kariotipovi, C - trake i položaj nukleolusnih organizatora (NOR) dve evropske vrste žaba, *Bufo bufo* i *Bufo viridis*, sa 7 jugoslovenskih lokaliteta. Kariotipski i hromozomalni markeri ovih vrsta poredeni su sa podacima koji se odnose na druge vrste i podvrste. Rezultati ukazuju da je raspored C-traka specifičane za ispitivane vrste. Kod vrste *B. bufo bufo* nađena je marker traka na petom hromozomu.

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