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Features of the karyotypes of *Pelophylax ridibundus* Pallas, 1771 and *Rana macrocnemis* Boulenger, 1885 (Amphibia: Ranidae) from Armenia

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Abstract. Chromosomal complements of *Pelophylax ridibundus* Pallas, 1771 from 9 localities (Northern, Central and South Armenia) and *Rana macrocnemis* Boulenger, 1885 from one locality (North-West Armenia) have been analyzed. The chromosome sets of *P. ridibundus* collected from 8 localities showed 2n=26, (10m+12sm+4st; NF=52). A secondary constriction has been observed in all studied individuals on the 10-th chromosome pair showing NOR-positive reaction. C-positive heterochromatin blocks have been observed on long arms of the 2-nd and 10-th pairs of chromosomes (7 localities). In addition, C-heterochromatin blocks have been found on interstitial regions of short arms of the 12-th pairs, as well as in telomeric regions of long arms of the 9-th pairs and on short arms of the 5-th pair in the frogs from 2 localities. The karyotype of *P. ridibundus* from populations near Ejmiatsin differs from other populations (2n=26, 12m+10sm+4st). Diploid number of chromosomes of *R. macrocnemis* was also 26 (8m+12sm+6st, NF=52). Blocks of C-positive heterochromatin have been revealed in telomeric parts of the 1-st, 2-nd (p), 3-rd (q), 4-th (q), 6-th, 9-th (p), 10-th (p,q) and 13-th (q) pairs, as well as in interstitial regions of the 1-st and 2-nd pairs of chromosomes. Intrapopulation and interpopulation geographic variations of karyotypes and C-heterochromatin banding patterns of *P. ridibundus* have been revealed. Karyotypically, morphotypes "macrocnemis" and "camerani" are closely related.

Key words: Amphibia, Ranidae, *Pelophylax*, *Rana*, karyotypes, C-positive heterochromatin blocks, chromosome polymorphism.

Introduction

In Armenia, the Marsh frog *Pelophylax ridibundus* (=*Rana ridibunda*) Pallas, 1771 inhabits all humid and freshwater biotopes at 850-2500 meters above sea level (Melkumyan, Sirunyan, 1988; Egiazaryan, 2008). The long-legged wood frog *Rana macrocnemis* Boulenger, 1885 demonstrates significant morphological variability, including two widespread morphotypes "macrocnemis" and "camerani" in the Caucasus Isthmus, in various parts of Anatolia and northern Iran (Tarkhnishvili et al., 2001; IUCN, 2008). *Rana macrocnemis* occurs in a very broad range of biotopes: in broadleaved and mixed forests, steppes, sub-alpine and alpine meadows at the altitude ranging from 1000-2500 meters above sea level in Armenia. (Melkumyan, Sirunyan, 1988; Egiazaryan, 2008). At the foot of the Jawajhet mountain range, where these frogs were caught, the "camerani" form was found (Tarkhnishvili et al., 2001). Few data about

the biology of these two species of the genera *Rana* Linnaeus, 1758 and *Pelophylax* Fitzinger 1883 living in Armenia have been published so far. It is known that *P. ridibundus* is characterized by intraspecific polymorphism in some morphometric features, such as body size and dorsal colour patterns (Melkumyan, Sirunyan, 1988; Manukyan, 2002). The biology of the two above mentioned species and mating calls of *P. ridibundus* have also been studied (Egiazaryan, 2008).

Presently, *P. ridibundus* is considered as a complex of species, based on molecular, genetic (Beerli, 1994; Beerli et al., 1996; Plötner, Ohst, 2001; Plötner et al. 2001; Plötner et al., 2008) and bioacoustic (Joermann et al., 1988; Schneider et al., 1992; Schneider, Sinsch, 1992, 1999) data. Karyotypes of species of the *P. ridibundus* complex have been studied from the populations of Western and Central Europe (Schmid, 1978; Mészarós, Bartos, 1978; Bucci et al., 1990; Spasić-Bošković et al., 1999), Greece (Tunner, Heppich, 1982), Ukraine (Suryadnaya, 2003), Russia (Birstein, 198l; Kaibeleva et al., 2004), Turkey (Alpagut, Falakali, 1995), China (Gang Wei et al., 1992) and Saudi Arabia (Al-Shehri, Al-Salech, 2005). Chromosome sets of brown frogs of the *Rana macrocnemis* complex have been described from populations from Russia and Georgia (Ivanov, Madyanov, 1973; Orlova et al., 1977; Birstein, 1984; Popov, Dimitrov, 1999). So far chromosome sets of Ranidae species from Armenia have not been studied at all.

The purpose of this study is to analyze the karyotypes and to evaluate the intraspecific and interspecific chromosome polymorphism of *P. ridibundus* and *R. macrocnemis*.

Material and Methods

Specimens were collected from 9 localities of Armenia (Table 1, Fig. 1) in 2003 and 2004.

Chromosome slides were prepared from bone marrow and spleen according to Haertel et al. (1974) and MacGregor, Varley (1986). C-banding was made as described in Sumner (1972) with some modifications. Ag-banding was done following the technique of Howell, Black (1978). Chromosome smears were observed under a «NU-2E» (K. Zeiss, Germany) microscope, with the 1125 magnification (90 x 12.5). Chromosome complements were analyzed in mitotic and meiotic stages of cell di-

Table 1. Collecting data.

Species	Number		Locality
	males	females	
Pelophylax ridibundus	3	1	Vicinity of the village Ranchpar
	1	2	Vicinity of Yerevan, canyon of the river Hrazdan
	4	1	Vicinity of the village Urcadzor
	2	4	Vicinity of the Ararat
	1	1	Vicinity of the village Shorzha, lakeside of Sevan
	2	2	Vicinity of the village Vohchaberd
	3	-	Vicinity of the village Garni, canyon of the river Azat
	-	1	Vicinity of the town Dilijan
	3	4	Vicinity of the Ejmiatsin
Rana macrocnemis form “camerani”	1	1	At the foot of the Jawajhet mountain range, vicinity of the Gukasyan

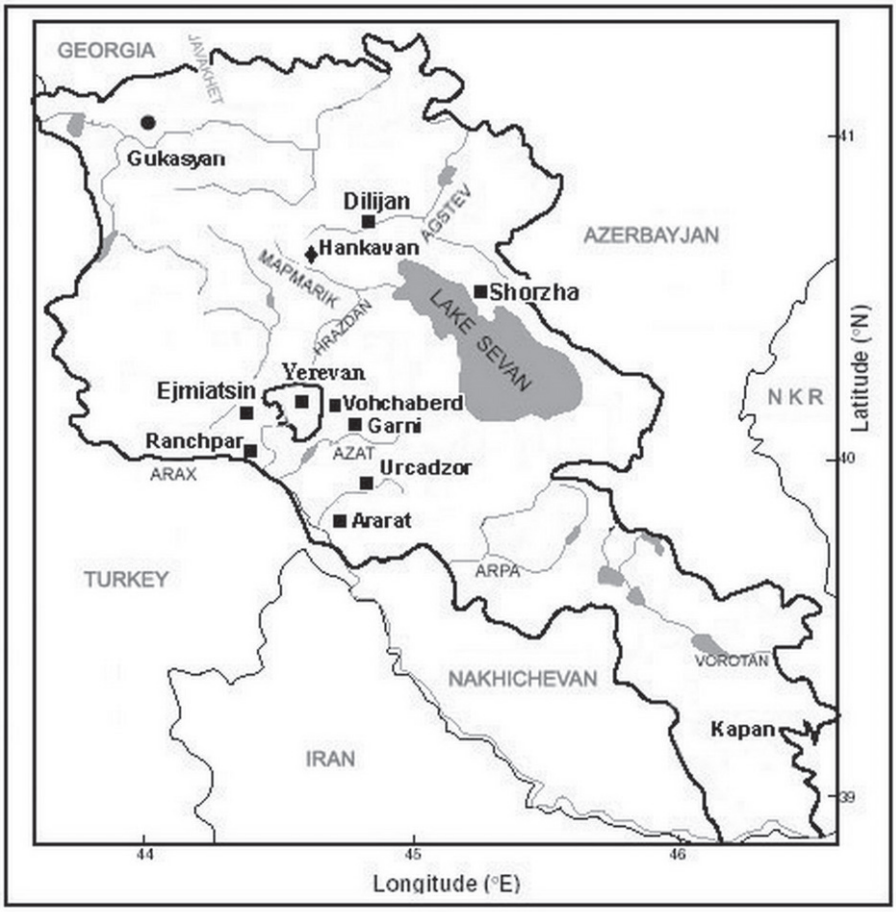

Fig. 1. Sample localities. *Pelophylax ridibundus* (black square – present study), (black rhomb – Schneider, Sinsch, 1999) and *Rana macrocnemis* form "camerani" (black circle – present study).

vision. Homologous chromosome pairs were identified according to their relative length (RCL) and the centromeric index (Cen. Ind) (Levan et al., 1964). Statistical analysis of chromosome pairs was performed using the program Statistica 6.0.

Results

Chromosome sets of the observed specimens are presented in Tables 2, 3 and in Figures 2, 3.

Pelophylax ridibundus

Diploid karyotypes of specimens from 8 localities in Armenia were similar in their chromosome numbers and morphology and consist of 26 chromosomes (NF=52). Chromosomal complements include 5 pairs of metacentric (m), 6 pairs of submetacentric (sm) and 2 pairs of subtelocentric (st) chromosomes (Tables 2 and 3). The chromosome set of frogs from a population near Ejmiatsin differs from other populations and possesses karyotypic formula of 12m+10sm+4st. A secondary constriction was demonstrated by Ag-bandings on the long arm of the submetacentric pair of chromosomes (10-th) in both sexes in all studied populations (Fig. 3, a). Differences between

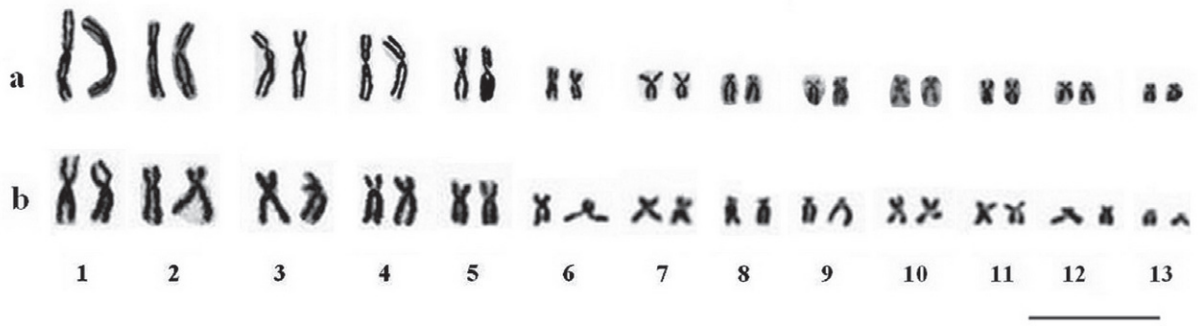

Fig. 2. Karyograms of *Pelophylax ridibundus* (**a** - Ejmiatsin), and *Rana macrocnemis* form "camerani" (**b**) from Armenia. Bar = 10 μm.

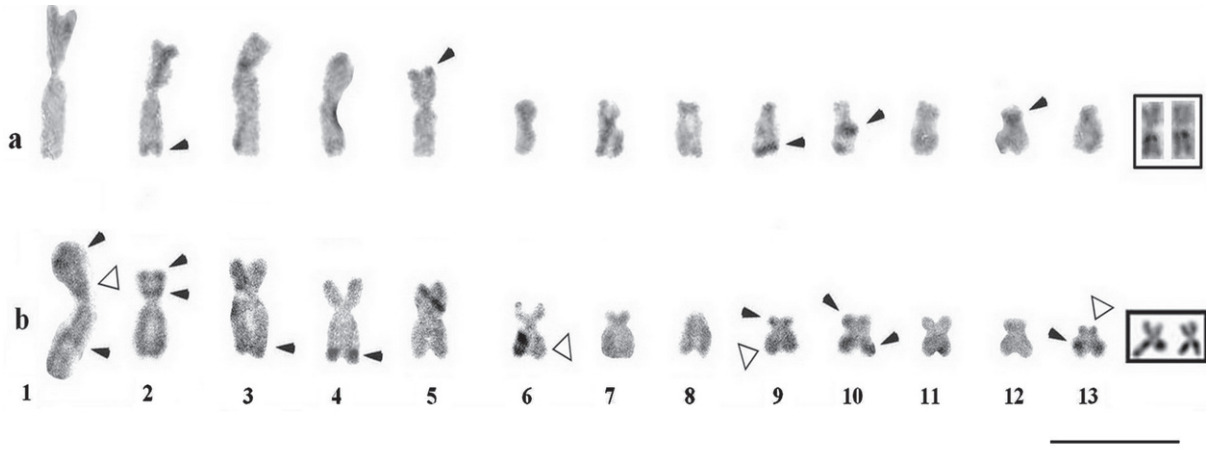

Fig. 3. Haploid banded chromosome sets of *Pelophylax ridibundus* (**a**) and *Rana macrocnemis* form "camerani" (**b**) from Armenia. Arrowheads indicate the variants of C-bands. White triangles indicate weakly marked C-banded regions. NOR-bearing chromosomes are shown in the frame. Bar = 10 μm.

chromosome sets of females and males have not been revealed.

C-positive heterochromatin blocks in karyotypes of frogs collected in Azat and Vohchaberd were revealed in the interstitial (i) regions of the long (q) arms of the 10-th chromosome pair and the short (p) arms of the 12-th, chromosome pair and telomeric regions of the long arms of the 2-nd and 9-th pairs and on the short arms of the 5-th pair (Table 3; Fig. 3, a). Blocks of C-heterochromatin in the chromosome sets of frogs collected from other 7 localities of Armenia were only observed on long arms of the 2-nd and 10-th pairs. Ag-banding revealed an argentophilous body localized on the submetacentric pair of chromosomes (10-th) of all males and females from all mentioned localities (Fig. 3, a).

Rana macrocnemis

The karyotype of the form "camerani" also includes 26 bi-armed chromosomes (NF=52; 8m+12sm+6st) (Fig. 2, b). Blocks of C-posi-

Table 2. Statistical data of chromosomes measurements. m – metacentric (Cen. Ind =50.0-37.5), sm – submetacentric (37.5-25.0), st – subtelocentric (25.0-12.5). STD- standard deviation, SE- standard deviation error. *The karyotype of *Pelophylax ridibundus* from 8 populations (without Ejmiatsin).

Pairs	*Pelophylax ridibundus* *				*R. macrocnemis*			
	Centromeric index			RCL	Centromeric index			RCL
	AVG	STD	SE	AVG±STD	AVG	STD	SE	AVG±STD
I	43.87	0.57	0.12	16.41±1.3	43.10	0.10	0.06	15.57±0.5
II	34.20	0.45	0.10	13.10±0.9	33.30	0.20	0.12	13.07±0.8
III	32.22	0.43	0.09	12.02±0.6	31.43	0.21	0.12	11.53±0.2
IV	43.00	0.34	0.07	11.50±0.8	30.23	0.21	0.12	11.13±0.1
V	33.32	0.63	0.13	9.22±1.8	38.60	0.10	0.06	10.27±0.7
VI	41.97	0.34	0.07	5.84±0.3	29.17	0.21	0.12	5.97±0.2
VII	41.90	0.56	0.12	5.38±0.5	33.37	0.21	0.12	5.67±0.3
VIII	20.81	0.50	0.11	5.14±0.4	21.83	0.15	0.09	5.43±0.4
IX	31.23	3.48	1.10	4.88±0.4	20.30	0.26	0.15	5.10±0.1
X	34.11	0.49	0.11	4.53±0.2	41.13	0.25	0.15	4.47±0.3
XI	40.46	7.18	2.17	4.18±0.3	44.63	0.25	0.15	4.1±0.8
XII	23.33	4.03	1.21	3.78±0.44	27.23	0.21	0.12	3.97±0.5
XIII	35.85	0.48	0.10	3.08±0.7	24.17	0.21	0.12	3.67±0.8

tive heterochromatin were revealed in telomeric regions of the 1-st, 2-nd (p), 3-rd (q), 4-th (q), 9-th (p), 10-th (p.q) and 13-th (q) pairs as well as in interstitial regions of the 2-nd pair of chromosomes (Fig. 3, b; Table 3). Weakly stained C-banding patterns were observed on the interstitial part of the arm of the 1-st pair, on the telomeric parts of the long arm of the 6-th pair and 9-th pair and, also, on the short arm of the 13-th pair (Fig. 3, b). The analysis of silver stained slides revealed NOR-bearing blokes in the 10-th chromosome pair (Fig. 3, b).

Discussion

Karyotypes of more than 300 studied species of the genera *Rana* Linnaeus, 1758 and *Pelophylax* Fitzinger 1883 are characterized by a standard chromosome number of 2n=26 (sometimes 22 or 24) including metacentric, submetacentric and subtelocentric chromosomes (Morescalchi, 1973, 1977; Ivanov, Madjanov, 1973; Schmid, 1978; Schmid et al., 1990; Orlova et al., 1978; Birstein, 1984; King, 1990; Kuramoto, 1990; Green, Borkin, 1993; Miura, 1995; Alpagut, Falakali, 1995; Spasič-Bošković, 1999; Al-Shehri, Al-Salech, 2005; Suryadnaya, 2003; Kaibeleva et al., 2004; Joshy et al., 2006; Mindrescui, Ghiorghyk, 2008).

The karyotype of *P. ridibundus* from different localities of Armenia has been examined for the first time within the framework of this study. It was demonstrated that diploid chromosomal set also equals to 26 and includes metacentric, submetacentric and subtelocentric morphological elements. The use of conventional Giemsa staining did not always display the secondary constriction, whereas Ag-banding of chromosomes did reveal it. Homologues of the 10-th pair in all the specimens studied (males and females) possess a secondary constriction on their long arms (Figs 2, a; 3, a).

The analysis of described karyotypes of *P. ridibundus* detected interpopulation polymor-

phism in Turkey (Alpagut, Falakali, 1995), Armenia (our data) and Russia (Kaibeleva et al., 2004) (Tables 3, 4). Usually these differences in the number of metacentric and submetacentric chromosomes in karyotypes of these lake frogs can be related to different stages of chromosome spiralization in homologues from the 6-th to 13-th pairs.

Comparison of chromosome sets of *P. ridibundus* from Armenia (8 populations), Turkey, Saudi Arabia, Ukraine, Russia, Central and South Europe, and China shows both similarities and distinctions. All karyotypes of *P. ridibundus* from different geographic regions studied are similar in their diploid chromosome numbers; in morphology of seven chromosome pairs: 1-st (m), 2-nd (sm), 3-rd (sm), 5-th (m), 6-th (m), 8-th (st), 13-th (sm); in the pair of submetacentric chromosome, bearing the secondary constriction (9-th - Russia, Ukraine, Turkey and 10-th -other populations), and in the 12-th pair of subtelocentric chromosomes (Armenia, Turkey, Saudi Arabia) at more accurate measurements (Table 4). Despite this, the chromosomal complements of *P. ridibundus* from the compared regions were different. Thus, interpopulation variations in numbers and morphology of metacentric, submetacentric and subtelocentric chromosomes of *P. ridibundus*, were demonstrated (Table 4). In the karyotypes of *P. ridibundus* from Saudi Arabia, Central Europe and Kabardino-Balkaria some of middle and small size chromosomes can be regarded as subtelocentrics (CI=21.9; CI=24.7) on the basis of our measurements. The observed differentiation in morphology of the 7-th to the 12-th chromosome pairs (Table 4) can be a result of different stages of chromosome spiralization (from meta- to submetacentric, from submeta- to subtelocentric). Three pairs of subtelocentic chromosomes in some populations of the lake frog may emerge as a result of chromosomal rearrangements (translocations) under the influence of different ecological conditions.

The interpopulation variations in different patterns of positions of C-positive heterochromatin regions were also revealed. The majority of the C-blocks (eleven pairs of the chromosomes) were observed in the karyotypes of *P. ridibundus* from populations of Central Europe (Schmid, 1978) and Armenian Azat and Vohchaberd (five pairs) (Fig.3, b; Table 3).

Interpopulation variations in C-positive heterochromatin regions have been described in some species of salamanders (genus *Hynobius* Tschudi, 1838; Ikebe et al., 1987), lizards (Capriglione et al., 1991, 1998; Yonenaga-Yassuda et al., 1996), snakes (genus *Vipera* Linnaeus, 1758; Aprea, et al., 2006) and rodents of the genera *Microtus* Schrank, 1798 (Kovalskaya et al., 1991) and *Peromyscus* Gloger, 1841 (Kaidanov, 1996). It is known that the intraspecific differences in the amount and distribution of heterochromatin found in taxa of the genus *Vipera* were taxonomically irrelevant (Aprea et al., 2006). However, these variations of heterochromatin proportions

Table 3 (see next two pages). The karyotype characteristics of *Pelophylax ridibundus* and *Rana macrocnemis*. a - Izmir, b - Beyşehir (Alpagut, Falakali, 1995); c - the frogs from flood-lands, d - the frogs from stagnant water (Kaibeleva et al., 2004). 2n - diploid number of chromosomes; m - metacentric, sm - submetacentric; st - subtelocentric chromosomes; Sec. con - second constriction; q - long arm of chromosome, p - short arm of chromosome, i - interstitial region of the chromosome arm. 12sm♦ - among submetacentric chromosomes 8th, 9th and 12th pairs can mark out as subtelocentric, 16sm▲ - among submetacentric chromosomes 8th and 9th pairs can mark out as subtelocentric, 16sm● - among submetacentric chromosomes 8th and 9th pairs can mark out as subtelocentric, ◊ – C-band positive heterochromatin, ¤ – heterochromatin, weakly stainable by C-banding 16sm, ■ – the chromosomes of 8th, 9th and 13th pairs can mark as subtelocentric.

Species	Population	2n	Sec. cons.	Sex chr	C-bnd	NOR	Karyotype formula	Sources
P. ridibundus	Armenia Wohchab., Azat	26	10q	-	2 q, 5 p, 9 q, 10q, 12p	10q	10m+12sm+4st	Authors data
	Yer., Arar., Dj., Ranch., Shr.,Urds.				2 q, 10q			
	Ejmiatsin				2,10q		12m+10sm+4st	
	Turkey	26	9q	XX XY			16m+4sm+6st (a)	Alpagut, Falakali, 1995
							15m+5sm+6st♀ (b) 14m+8sm+4st♂	
	Saudi Arabia	26	10q	XX XY		10q	14m+12sm♦	Al-Shehri, Al-Salech, 2005
	Yugoslavia (currently Serbia), Macedonia	26	10q	XX XY	3p	10q	8m+14sm+4st	Spasić-Bošković et al., 1999
	Poland	26	10q		2p		10m+12sm+4st?	Bucci et all, 1990
	Greece	26			2p		?	Tunner et al., 1982
	Cent. Europe	26	10q		◊(1, 2p,3q 4i, 5i,7p, 9p, 10qi, 11p) ¤(4qi, 5p, 8qi, 13q)	10q	10m+16sm▲	Schmid, 1978
	Hungary	26					12 m +10sm +4st	Mészarós, Bartos, 1978
	Kabardino-Balkaria, Ukraine, Georgia	26	10q	-	-		10m+16sm•	Ivanov, Madjanov, 1973
	Saratov Region Russia	26	9q				8m +14sm + 4st [c]	Kaibeleva et al., 2004
							10m+12sm +4st [d]	
	Ukraine	26	9q	-	-	-	8m+14sm+4st	Suryadnaya, 2003
	China	26		-	-	-	14m+12sm	Gang Wei et al., 1992

Table 3 (continuation).

R. macrocnemis, form "camerani"	Armenia	26	10	-	◊(1, 2pi, 3q,4q 9p, 10pq, 13q) ¤(1, 6q,9q 13p)	10	8m+12sm+6st	Authors data
	Unknown		-	-	-	-	12m+8sm+6st	Popov, Dimitrov, 1999
	Georgia		-	-	-	-	12m+10sm+4st	Ivanov, Madyanov, 1973
R. macrocnemis, form "macrocnemis"	Kabardino-Balkaria; Karachai-Cherkess	26	-	-	-	-	10m+12sm+4st	Ivanov, Madyanov, 1973
	Russia Caucasus		10	-	-	10	10m+16sm■	Birstein, 1984

might play an adaptive role. These variations can provide a genotypic adaptive advantage to changing environmental conditions faster than mutational processes (Prokofeva-Belgovskaya, 1986).

According to the literature, C-heterochromatin is concentrated in the centromere region in many species of the family Ranidae (Schmid, 1978; Miura, 1995). Telomeric bands were seen in the short arm of the 3-rd and 5-th chromosomal pair of *Pelophylax ridibundus*, *P. esculentus* Linnaeus, 1758 and *P. lessonae* Camerano, 1882 (Heppich, 1978). The comparison of C-banding patterns in karyotypes of *P. ridibundus* from Armenia (our data) and Central Europe (Heppich, 1978; Schmid, 1978) showed that two pairs of chromosomes (5-th and 10-th) bear similar C-positive blocks. It is known that constitutive heterochromatin in the genera *Rana* and *Pelophylax* reacts variably to alkaline pretreatment and $Ba(OH)_2$ treatment (Schmid, 1978). The fact that the C-heterochromatin blocks are to be detected only in telomeric regions of chromosomal arms (Armenia) or on one chromosome pair might be accounted for by the methodical features of the chromosome slide preparation. However, the case of the C-heterochromatin appearance on short arms of the 3-rd pair (Spasič-Bošković et al., 1999) could be explained by pericentric inversion.

Based on molecular, genetic (Beerli, 1994; Beerli et al., 1996; Plötner, Ohst, 2001; Plötner et al. 2001) and bioacoustic (Joermann et al., 1988; Schneider, Sinsch, 1992, 1999) data some taxonomical changes occurred within the *P. ridibundus* comlex. Thus, now in Russia and Ukraine P. *ridibundus* is described (Ananjeva et al., 1998), in Eastern Turkey - *P. caralitanus* (= *R. caralitana*) Arikan, 1988 (Plötner, 2005; Ayaz et al., 2006), in Central and South-Western Europe – *Rana fortis* Boulenger, 1884

(Plötner, 2005; Plötner et al., 2008).

In light of the analysis of chromosomal sets (Schmid, 1978; Mészarós, Bartos, 1978; Bucci et al., 1990; Spasič-Bošković et al., 1999) and modern notions on taxonomic status and zoogeographical distribution of species of the *P. ridibundus* complex, the slight differences between the karyotypes of frogs from Central and South-Western Europe (Serbia and Macedonia, Hungary) can be accounted by a different degree of chromosome spiralization (from meta- to submetacentric, from submeta- to subtelocentric) within the karyotype of *R. fortis*. Taking into consideration the fact that *R. fortis* inhabits Europe (Plötner, 2005; Plötner et al., 2008) and *P. ridibundus* inhabits Russia and Ukraine (Ananjeva et al., 1998), the karyological differences of these species in 2 pairs of chromosomes (m/sm) (Tables 3, 4) can be noted as interspecific. However, these chromosomal set distinctions at the morphological level (metacentric/ submetacentric chromosomes) can most likely be caused by the degree of chromosome spiralization rather than by chromosomal reorganizations.

According to Sinsch, Schneider (1999) *P. ridibundus* inhabits Armenia. The occurrence of *P. bedriagae* Camerano, 1882 in Armenia (Ananjeva et al., 1998) is doubtful, since authors (Ananjeva et al., 1998; Plötner, 2005) disagree on the frog's distribution. The analysis of chromosomal complements of *P. ridibundus* from Armenia and Ukraine showed the differences in morphology of the three pairs of chromosome (Table. 4), which can be accounted by various degrees of chromosome spiralization (m/sm). These karyotype distinctions can be noted as intraspecific.

The comparison of the karyotypes of lake frogs from Armenia (*P. ridibundus*) and Eastern Turkey (Beyşehir and Izmir populations (Alpagut, Falakali, 1995)) (*P. caralitanus*) (Plötner, Ohst, 2001; Plötner, 2005; Ayaz et al., 2006) shows the differences in three chromosome pairs (m, sm, st) (Table. 4). These differences in the chromosome sets might be interspecific and can arise as a result of chromosomal arrangements in the karyotype of *P. caralitanus*.

Comparison of chromosome sets of lake frogs in Armenia (*P. ridibundus*) and Europe (*R. fortis*) (Schmid, 1978; Mészarós, Bartos, 1978; Bucci et al., 1990; Spasič-Bošković et al., 1999; Plötner, 2005; Plötner et al., 2008) reveals the differences in the number of chromosomal pairs and C-banding patterns localization, which can be explained as interspecific.

The data on the distribution of *P. bedriagae* in Saudi Arabia (Sinsch, Schneider, 1999; Plötner, 2005) are questionable, since authors disagree on the frog's distribution. Based on abovementioned, the chromosome sets of lake frogs (*P. ridibundus*) inhabiting in Saudi Arabia (Sinsch, Schneider, 1999; Al-Shehri, Al-Salech, 2005) and Armenia differ in three pairs of chromosomes (Table 4). Only one pair (9-th) among the differing pairs can be accounted for by the formation of chromosomal reorganizations (sm/st). Other pairs (7-th, 12-th) differ at the metacentric and submetacentric morphological elements level. Taking into account the fact that the same species (*P. ridibundus*) inhabits both in Armenia and Saudi Arabia, distinctions in chromosomal complements of *P. ridibundus* refers only to interpopulation chromosomal polymorphism.

The analysis of the studied karyotypes of lake frogs inhabiting in Saudi Arabia (*P. ridibundus*) and in Western Turkey (*P. caralitanus*) also demonstrated the difference in four pairs of chromosomes (Table 4). Taking into account the taxonomic status of these frogs and chromosome sets differentiation (m/st pairs), the differences between karyotypes of lake frogs in Turkey and Saudi Arabia can also

Table 4. The chromosome features of *Pelophylax ridibundus* and *Rana macrocnemis* from different localities (• - NOR bearing chromosomes, sm* - pairs can mark out as subtelocentric).

Sources	Chromosome pairs												
	I	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*	*IX*	*X*	*XI*	*XII*	*XIII*
Pelophylax ridibundus group													
Armenia (8 populations)	m	sm	sm	m	sm	m	m	st	sm	•sm	m	st	sm
Ukraine (Suryadnaya, 2003)	m	sm	sm	sm	m	m	m	st	•sm	st	sm	sm	sm
Saratov Region (Kaibeleva et al., 2004)	m	sm	sm	m	m	m	sm	st	•sm	st	sm	sm	sm
Kabardino-Balkaria Ukraine, Georgia (Ivanov, Madjanov, 1973)	m	sm	sm	m	sm	m	m	sm*	sm*	•sm	m	sm	sm
Hungary (Mészarós, Bartos, 1978)	m	sm	sm	m	m	m	m	st	st	sm	m	sm	sm
Cent. Europe (Schmid, 1978)	m	sm	sm	m	m	sm	m	sm*	sm*	•sm	m	sm	sm
Yugoslavia (cur. Serbia), Macedonia (Spasić-Bošković et al.,1999)	m	sm	sm	sm	m	m	st	sm	m	•sm	st	sm	sm
Turkey (Beyşehir) (Alpagut, Falakali, 1995)	m	sm	m	m	m	m	m	st	•sm	m	m	st	st
Saudi Arabia (Al-Shehri, Al-Salech, 2005)	m	sm	m	m	m	m	m	sm*	sm*	•sm	m	sm*	sm
China (Gang Wei et al., 1992)	m	m	sm	m	m	m	m	sm	sm	sm	m	sm	sm
Rana macrocnemis group													
Armenia	m	sm	sm	sm	m	sm	st	st	sm	•m	m	sm	st
Georgia (Ivanov, Madjanov, 1973)	m	sm	sm	sm	m	m	sm	st	st	m	m	sm	m
Popov, Dimitrov, 1999	m	sm	sm	m	m	sm	m	st	st	m	m	sm	st
Russia, Caucasus (Birstein, 1984)	m	sm	sm	m	sm	sm	m	sm*	sm*	•m	m	sm	sm*
Kabardino-Balkaria, Karachai-Cherkessia (Ivanov, Madjanov, 1973)	m	m	sm	sm	m	sm	m	sm	m	st	sm	sm	st

be interspecific. Despite this, karyological differences in frogs of the *P. ridibundus* complex are not comparable with interspecific differences in other green frog species (Heppich, 1978; Schmid, 1978; Belcheva et al., 1985; Miura, 1995; Miura et al., 1997), as the morphological elements of the karyotype vary both among populations of *P. ridibundus* as well as among the species of *P. ridibundus* complex. It is known that the several techniques features, such as C-banding patterns, fluorescence replication banding patterns (DAPI and FISH) are used for an accurate distinction of the species of green and brown frogs (Schmid, 1978; Miura, 1995; Miura et al., 1997; Picariello et al., 2002). Hence, those should also be used for the final confirmation of differences in the frog species within the *P. ridibundus* complex.

The comparative analysis of the karyotypes of frogs belonging to the complex of *R. macrocnemis* from Armenia (our data), Georgia (Ivanov, Madjanov, 1973), (Popov, Dimitrov, 1999) and form "macrocnemis" from Russia (Birstein, 1984; Ivanov, Madjanov, 1973) revealed their similarities on chromosomal pairs № 1, 2, 3, 4, 5, 6, 7 and 12 (Table 4). The cardinal differences of the chromosomal sets of "camerani" and "macrocnemis" forms relate to the chromosomal pairs № 8, 9, 10, 11 and 13 (Table 4). Interpopulation differentiations in number and morphology of the karyotypes elements of two forms of *R. macrocnemis* may relate to different degrees of chromosomes spiralization (m to sm) and, also, to be a result of chromosomal rearrangements (m to st; sm to st) under different ecological conditions. The analysis of the literature data of the external morphology, blood serum protein electrophoresis, allozymes and mtDNA, DNA sequence study (Ischenko, 1987; Tarkhnishvili et al., 2001; Veith et al., 2003; Çevik et al., 2006) of the frogs of the forms "camerani" and "macrocnemis" did not show significant qualitative interspecific differences and led authors to consider "macrocnemis" and "camerani" as morphotypes. Thus, these brown frogs are chromosomally more closely related as well.

Comparison of the chromosome sets of *Pelophylax ridibundus* and *Rana macrocnemis* from Armenia detects the similarities in their diploid number of chromosomes (26), in the morphology of the chromosome pairs: 1-st, 2-nd, 3-rd, 5-th, and two pairs of subtelocentric chromosomes (8-th and 12-th/13-th) (Fig. 2 a, b; Table 4). Besides that, at comparison of distribution of C-banding patterns in the chromosome pairs of *P. ridibundus* (Schmid, 1978) and *R. macrocnemis* form "camerani" the similarities on the 1-st (p.t.), 2-nd (q.t.), 3-rd (p.t.; p.i.) and 13-th (q.t.) pairs were shown (Fig. 3, a, b). The distinctions between the species are displayed in the different morphology of the several chromosome pairs (4-th, from 6-th to 11-th), in the secondary constriction-bearing chromosome pairs, in the morphology of NOR-positive regions bearing chromosome pairs and C-positive heterochromatin blocks localizations in the karyotype (Table 4).

Comparison of the chromosomal data of *P. ridibundus* with other species of the green frogs (Heppich, 1978; Schmid, 1978; Miura, 1995; Miura et al., 1997) and some species of brown frogs (Orlova et al., 1978; Birstein, 1984; Green, Borkin, 1993; Popov, Dimitrov, 1999) with data from this study confirms the close similarity of several chromosome pairs by size and morphology. On the whole, the interspecific differences in karyotypes of species of *Rana* and *Pelophylax* are manifested in the distinctions of late replication banding patterns (Miura, 1995; Miura et al., 1997), fluorescence replication banding patterns (Picariello et al., 2002) and in the locations of C-heterochromatin blocks (Schmid, 1978; Miura, 1995; Miura et al., 1997; present study).

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References

Alpagut N., Falakali B. 1995. Karyotype analysis of two *Rana ridibunda* (Ranidae; Anura) populations in Turkey // *Israel J. Zool.* 41: 523-531.

Al-Sherhi A.H., Al-Salech A.A. 2005. Karyotype of amphibians in Saudia Arabia 1: The karyotype of *Rana ridibunda* // *J. Biol. Sci.* 5(3): 335-338.

Ananjeva N. B, Borkin L.Y., Darevsky I.S., Orlov N. L. 1998. The encyclopedia of nature of Russia. Amphibians and Reptiles. Moscow. 576 p. (In Russian).

Aprea G., Gentilli A., Zuffi M.A.L., Odierna G. 2006. The karyology of *Vipera aspis*, *V. atra*, *V. hugyi*, and *Cerastes vipera* // *Amphibia-Reptilia.* 27: 113-119.

Ayaz D., C. V.Tok, A. Mermer, M. Tosunoğlu, M. Afsar, Çiçek K. 2006. A new locality for *Rana ridibunda caralitana* Arıkan, 1988 (Anura: Ranidae) in the Central Anatolia // *J. Fish. Aqw. Sci.* 23 (1-2): 181-183.

Belcheva R., Michallova P., Sofianidou T. 1985. Karyological studies on *Rana epeirotica* and *Rana ridibunda* (Anura, Amphibia) from Greece // *Dokl. Bolgarian Akad. Nauk.* 38 (10): 1387-1390.

Beerli P. 1994. Genetic isolation and calibration of an average protein clock in western Palearctic water frogs of the Aegean region. PhD Dissertation, Universität Zürich.

Beerli P., Hotz H., Uzzell T. 1996. Geologically dated sea barriers calibrate a protein clock for Aegean water frogs // *Evolution.* 50: 1676-1687.

Birstein V.J., 198l. Specific differential staining patterns of chromosomes in the Anura, some species of the genera *Rana* and *Bufo* taken as an example // *Zool. Zh.* 60: 246-256. (In Russian).

Birstein V.J. 1984. Localization of NORs in karyotypes of four Rana species // *Genetica.* 64: 149-154.

Bucci S., Ragghianti M., Mancino G., Berger L., Hotz H. and Uzzell T. 1990. Lampbrush and mitotic chromosomes of the hemiclonally reproducing hybrid *Rana esculenta* and its parental species // *J. Exp. Zool.* 255: 37-56.

Capriglione T., Cardone A., Odierna G., Olmo E. 1991. Evolution of a centromeric satellite DNA and phylogeny of lacertid lizards // *Comp. Biochem. Physiol.* 100B: 641-645.

Capriglione T., De Santo M.G., Morescalchi M.G., Odierna G., Olmo E. 1998. Organization of an alphoid-like satellite DNA sequence in the genome of the lacertid lizard, *Lacerta graeca* // *J. Mol. Evolut.* 46: 240-244.

Çevik I., E., H., Arıkan, U., Kaya, M., K., Atatür. 2006. Comparative morphological and serological studies of three Anatolian Mountain frogs, *Rana macrocnemis*, *R. camerani* and *R. holtzi* (Anura, Ranidae) // *Amphibia-Reptilia.* 27: 63-71.

Egiazaryan E.M. 2008. Fauna and ecology of the amphibians from Armenia. Abstract of PhD Dissertation. Institute of Zoology. Yerevan. 44 p. (In Russian).

Gang Wei, Ning Xu, Dejun Li, Min Wu. 1992. Kariotypes of two *Rana* from Xinjiang, China // *Asiatic Herpetological Res.* 4: 141-145.

Green D., Borkin L. 1993. Evolutionary relationships of eastern Palearctic brown frogs, genus *Rana*: parafyly of the 24-chromosome species group and the significance of chromosome number change // *Zool. J. Linnean Soc.* 109: 1-25.

Haertel T.D., Owezarzak A., Storm R.M. 1974. A comparative study of the chromosomes from five species of the genus *Rana* (Amphibia, Salentia) // *Copeia.* 1: 109-114.

Heppich S. 1978. Hybridogenesis in *Rana esculenta*: C-band karyotypes of *Rana ridibunda*, *Rana lessonae* and *Rana esculenta* // *Z. Zool. Syst. Evolut.* 16: 27-39.

Howell W.M., Black D.A. 1978. A rapid technique for producing silver-stained nucleolus organizer regions and trypsin-Giemsa bands on human chromosomes // *Hum. Genet.* 43: 53-56.

Ikebe C., Aoki K-I., Kohno S.-I. 1987. Karyotype analysis of two Japanese salamanders, *Hynobius nebulosus* (Schlegel) and *Hynobius dunni* Tago, by means of C-banding // *Zool. Sci.* 4: 621-626.

Ishchenko V.G. 1987. The level of morphological similarity between the populations the Caucasian brown frog, *Rana macrocnemis* Blgr. // *Proc. Zool. Inst. Acad Sci. USSR.* 158: 100-104. (In Russian).

Ivanov V.G., Madyanov N.N. 1973. The comparative karyology of frogs of the genus *Rana* // *Tsitologia.*

16: 920-927 (In Russian).

IUCN. 2008. IUCN Red List of Threatened Species. (www.iucnredlist.org. on May 04, 2009).

Joermann G., Baran I., Schneider H. 1988. The mating call of *Rana ridibunda* (Amphibia: Anura) in western Turkey: Bioacoustic analysis and taxonomic consequences // *Zool. Anz.* 220: 225-232.

Joshy S.H., Kuramoto M., Sreepada K.S. and Rahiman M.A. 2006. Karyotypic variations in three Indian species of the genus *Rana* (Anura: Ranidae) from the Western Ghats, India // *Cytologia.* 71(1): 63-68.

Kaidanov L.Z. 1996. Genetics of the populations. Moskow. 264 p. (In Russian).

Kaybeleva E.I., Zavyalov E.V., Tabachaishin V.G. 2004. Ecologo-caryological peculiarities of the marsh frogs on the north of the Lower Volga region // *Povolzskii Ecol. J.* 3: 318-319. (In Russian).

King M. 1990. Amphibia // John B. (Ed.). *Animal Cytogenetics.* 4. 241 p.

Koval'skaya Yu.M., Aniskin V.M., Kartavseva I.V. 1991. Geographic variation of C-heterochromatine in Microtus fortis (Rodentia, Cricetidae) // *Zool. Zh.* 70(12): 97-103. (In Russian).

Kuramoto M. 1990. A list of chromosome numbers of anuran amphibians // *Bull. Fukuoka Univ.* (III). 39: 83-127.

Levan A., Fredga K., Sandberg A.A. 1964. Nomenclature for centromeric position on chromosomes // *Hereditas.* 52: 201-220.

MacGregor H.C., Varley J.M. 1986. Working with animal chromosomes. Moscow. 272 p. (In Russian).

Manukyan A. 2002. The comparative characteristic of the some morphometric features of the *Rana ridibunda* Pall. Diploma Thesis, Yerevan State Pedagogical Univ. Yerevan. 20 p. (In Armenian).

Melkumyan L.S., Sirunyan A.S. 1988. The maximal size of the amphibians populations inhabiting in the different altitude above sea level // *Biol. J. Armenia.* 41(8): 707-709. (In Russian).

Mészarós B., Bartos L.A. 1978. *Rana esculenta*-formakör három magyarország alakjának kariológiai feldolgozása // *Acta Biol. Debres.* 15: 239-256.

Mindrescui C.G., Ghiorghyk G. 2008. The preliminary cytogenetic investigations in *R. ridibunda* Pall. and *R. esculenta* L. from the north of Moldavia // *Ann. Stcf. Univ.* (Genet. Mol. Biol.). 9: 65-69.

Miura I. 1995. The late replication banding patterns of chromosomes are highly conserved in the genera *Rana*, *Hyla* and *Bufo* (Amphibia: Anura) // *Chromosoma.* 103: 567-574.

Miura I., Ohtani H., Nakamura M., Saitoh K., 1997. Fluorescence replication banding of frog chromosomes // *Cell. Mol. Life. Sci.* 53: 73-77.

Morescalchi A. 1973. Cytotaxonomy of the Amphibia, (pp. 233-347) // Chiareli A., Capanna B. (Eds). Cytotaxonomy and Vertebrate Evolution. London-New York. 348 p.

Morescalchi A. 1977. New developments in vertebrate cytotaxonomy. I. Cytotaxonomy of the amphibians // *Genetica.* 50: 179-193.

Orlova V.F., Bakharev V.A., Borkin L.J. 1977. Karyotypes of brown frogs of Eurasia and taxonomic analysis of karyotypes of the group // *Proc. Zool. Inst Acad. Sci. USSR.* 74: 51-103. (In Russian).

Picarello O., Feliciello I., Bellinello R., Chinali G. 2002. S1 satellite DNA as a taxonomic marker in brown frogs: molecular evidence that *Rana graeca* graeca and *Rana graeca italica* are different species // *Genome.* 45(1): 63-70.

Plötner J. 2005. Die westpaläarktischen Wasserfrösche: von Märtyren der Wissenschaft zur biologischen Sensation.. Bielefeld. 160 p.

Plötner, J., Ohst, T. 2001. New hypotheses on the systematics of the western Palearctic water frog complex (Anura, Ranidae) // *Mitt. Mus. Natur. kd. Berlin.* (Zool.). 77: 5-21.

Plötner J., Ohst T., Böhme W., Schreiber R. 2001. Divergence in mitochondrial DNA of Near Eastern water frogs with special reference to the systematic status of Cypriote and Anatolian populations (Anura, Ranidae) // *Amphibia-Reptilia.* 22: 397-412.

Plötner J., Uzzell T, Beerli P., Spolsky C., Ohst T., Litvinchuk S. N., Guex G.-D., Reyer H.-U., Hotz H. 2008. Widespread unidirectional transfer of mitochondrial DNA: a case in western Palaearctic water frogs // *J. Evol. Biol.* 21: 668–681.

Popov P., Dimitrov B. 1999. Karyotypes study of *Rana camerani* and comparisons with the other 26-chromosome European brown frog species (Amphibia, Anura) // *Cytobios.* 97: 13-22.

Prokofeva-Belgovskaya A.A. 1986. Heterochromatic regions of the chromosomes. Moskow. 431 p. (In Russian).

Spasić-Bošković O., Krizmanić I., Vujošević M. 1999. Population composition and genetic variation of water frogs (Anura; Ranidae) from Yugoslavia // *Caryologia.* 52(1-2): 9-20.

Schmid M. 1978. Chromosome banding in amphibian. II. Constitutive heterochromatin and nucleolus or-

ganizer regions in Ranidae, Microhylidae and Rhacophoridae // *Chromosoma.* 68: 131-148.

Schmid M., Steinlein C., Nanda I., Epplen J.T. 1990. Chromosome banding in Amphibia, (pp. 21-45) // Olmo E. (Ed.). *Cytogenetics of Amphibians and Reptiles.* Basel. 201 p.

Schneider H., Sinsch U., Nevo E. 1992. The lake frogs in Israel represent a new species // *Zool. Anz.* 228: 97-106.

Schneider H., Sinsch U. 1999. Taxonomic reassessment of Middle Eastern water frogs: Bioacustic variation among populations considered as *Rana ridibunda*, *R. bedriagae* or *R. levantina* // *J. Zool Syst. Evolut. Res.* 37: 57-65.

Sinsch U., Schneider H. 1999. Taxonomic reassessment of Middle Eastern water frogs: Morphological variatioamong populations consedered as *Rana ridibunda*, *R. bedriagae* or *R. levantina* // *J. Zool Syst. Evolut. Res.* 37: 67-73

Sumner A.T. 1972. A simple technique for demonstrating centromeric heterochromatin // *Exp. Cell. Res.* 75: 304-306.

Suryadnaya N.N. 2003. The data on caryology of green frogs (*Rana ridibunda*, *R. lessonae*, *R. esculenta*) from Ukraine // *Vestnik Zoologii.* 37(1): 33-40. (In Russian).

Tarkhnishvili D., Hille A., Bohme W. 2001. Humid forest refugia, speciation and secondary introgression between evolutionary linesges: differentiation in a Near Eastern brown frog, *Rana macrocnrmis* // *Biol. J. Linnean Soc.* 74: 141-156

Tunner H.G., Heppich S. 1982. A genetic analysis frogs from Greece: evidence for the existence of a cryptic species // *J. Zool. Syst. Evol.* 20: 209-223.

Veith M., Schmidtler F., Kosuch J., Baran I., Seitz A. 2003. Paleoclimatic changes explain Anatolian mountain frog evolution: a test for alternating vicariance and dispersal events // *Mol. Ecol.* 12: 185-189.

Yanenaga-Yassuda Y., Mori L., Chu T.H., Rodriques M.T. 1996. Chromosomal banding patterns in the eyelid-less microteiid radiation: *Procellosaurinus* and *Vanzosaura* (Squamata, Gymnophthalmidae) // *Cytogenet. Cell Genet.* 74: 203-210.

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