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; Mészáros, Bartos, 1978; Bucci et al., 1990; Spasić-Bošković et al., 1999), Greece (Tunner, Heppich, 1982), Ukraine (Suryadnaya, 2003), Russia (Birstein, 1981; 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>
<thead>
<tr>
<th>Species</th>
<th>Number</th>
<th>Localities</th>
</tr>
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<tbody>
<tr>
<td><em>Pelophylax ridibundus</em></td>
<td>3 males</td>
<td>Vicinity of the village Ranchpar</td>
</tr>
<tr>
<td></td>
<td>1 females</td>
<td>Vicinity of Yerevan, canyon of the river Hrazdan</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Vicinity of the village Urcadzor</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Vicinity of the Ararat</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Vicinity of the village Shorza, lakeside of Sevan</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Vicinity of the village Vohchaberd</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Vicinity of the village Garni, canyon of the river Azat</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>Vicinity of the town Dilijan</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Vicinity of the Ejmiatsin</td>
</tr>
<tr>
<td><em>Rana macrocnemis</em></td>
<td>1 males</td>
<td>At the foot of the Jawajhet mountain range,</td>
</tr>
<tr>
<td>form “camerani”</td>
<td>1 females</td>
<td>vicinity of the Gukasyan</td>
</tr>
</tbody>
</table>

**Table 1. Collecting data.**
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
chromosome sets of females and males have not been revealed.

C-positive heterochromatin blocks in karyotypes of frogs collected in Azat and Vochhaberd 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. Agbanding 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-
Features of the karyotypes of Pelophylax ridibundus and Rana macrocnemis

Table 2. Statistical data of chromosomes measurements. m – metacentric (Cen. Ind =50.0-37.5), sm – sub-metacentric (37.5-25.0), st – subtelocentric (25.0-12.5). STD- standard deviation, SE- standard deviation error.

<table>
<thead>
<tr>
<th>Pairs</th>
<th>Pelophylax ridibundus *</th>
<th>R. macrocnemis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Centromeric index</td>
<td>RCL</td>
</tr>
<tr>
<td></td>
<td>AVG ±STD</td>
<td>STD AVG ±STD</td>
</tr>
<tr>
<td>I</td>
<td>43.87 ±0.57</td>
<td>0.12 16.41±1.3</td>
</tr>
<tr>
<td>II</td>
<td>34.20 ±0.45</td>
<td>0.10 13.10±0.9</td>
</tr>
<tr>
<td>III</td>
<td>32.22 ±0.43</td>
<td>0.09 12.02±0.6</td>
</tr>
<tr>
<td>IV</td>
<td>43.00 ±0.34</td>
<td>0.07 11.50±0.8</td>
</tr>
<tr>
<td>V</td>
<td>33.32 ±0.63</td>
<td>0.13 9.22±1.8</td>
</tr>
<tr>
<td>VI</td>
<td>41.97 ±0.34</td>
<td>0.07 5.84±0.3</td>
</tr>
<tr>
<td>VII</td>
<td>41.90 ±0.56</td>
<td>0.12 5.38±0.5</td>
</tr>
<tr>
<td>VIII</td>
<td>20.81 ±0.50</td>
<td>0.11 5.14±0.4</td>
</tr>
<tr>
<td>IX</td>
<td>31.23 ±3.48</td>
<td>1.10 4.88±0.4</td>
</tr>
<tr>
<td>X</td>
<td>34.11 ±0.49</td>
<td>0.11 4.53±0.2</td>
</tr>
<tr>
<td>XI</td>
<td>40.46 ±7.18</td>
<td>2.17 4.18±0.3</td>
</tr>
<tr>
<td>XII</td>
<td>23.33 ±4.03</td>
<td>1.21 3.78±0.44</td>
</tr>
<tr>
<td>XIII</td>
<td>35.85 ±0.48</td>
<td>0.10 3.08±0.7</td>
</tr>
</tbody>
</table>

*The karyotype of Pelophylax ridibundus from 8 populations (without Ejmiatsin).

The karyotype of Pelophylax 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 Pelophylax ridibundus detected interpopulation polymor-
Phylogeny 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 subtelocentric 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 intraspecif 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

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Table 3 (see next two pages). The karyotype characteristics of *Pelophylax ridibundus* and *Rana maculosa*. 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 - secondary 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 out as subtelocentric.
<table>
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<th>Species</th>
<th>Population</th>
<th>2n</th>
<th>Sec. cons.</th>
<th>Sex chr</th>
<th>C-bnd</th>
<th>N O R</th>
<th>Karyotype formula</th>
<th>Sources</th>
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<tbody>
<tr>
<td><em>P. ridibundus</em></td>
<td>Armenia Wohchab., Azat</td>
<td>26</td>
<td>10q</td>
<td>-</td>
<td>2 q, 5 p, 9 q, 10q, 12p</td>
<td>10q</td>
<td>10m+12sm+4st</td>
<td>Authors data</td>
</tr>
<tr>
<td></td>
<td>Yer., Arar., Dj., Ranch., Shr., Urd.</td>
<td></td>
<td>10q</td>
<td>-</td>
<td>2 q, 10q</td>
<td></td>
<td>12m+10sm+4st</td>
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</tr>
<tr>
<td></td>
<td>Ejmiatsin</td>
<td>26</td>
<td>2,10q</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Turkey</td>
<td>26</td>
<td>9q</td>
<td>XX</td>
<td>10q</td>
<td>15m+5sm+6st (a)</td>
<td>15m+5sm+6st+ (b)</td>
<td>14m+8sm+4st+</td>
</tr>
<tr>
<td></td>
<td>Saudi Arabia</td>
<td>26</td>
<td>10q</td>
<td>XX</td>
<td>10q</td>
<td>14m+12sm+</td>
<td></td>
<td>Al-Shehri, Al-Salech, 2005</td>
</tr>
<tr>
<td></td>
<td>Yugoslavia (currently Serbia), Macedonia</td>
<td>26</td>
<td>10q</td>
<td>XX</td>
<td>3p</td>
<td>8m+14sm+4st</td>
<td></td>
<td>Spasić-Bosković et al., 1999</td>
</tr>
<tr>
<td></td>
<td>Poland</td>
<td>26</td>
<td>10q</td>
<td>2p</td>
<td></td>
<td>10m+12sm+4st</td>
<td></td>
<td>Bucci et all, 1990</td>
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<tr>
<td></td>
<td>Greece</td>
<td>26</td>
<td>2p</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tunner et al., 1982</td>
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<tr>
<td></td>
<td>Cent. Europe</td>
<td>26</td>
<td>10q</td>
<td></td>
<td>5'1, 2p, 3q, 4i, 5i, 7p, 9p, 10q, 11p) a'(4q, 5p, 8q, 13q)</td>
<td>10q</td>
<td>10m+16sm+</td>
<td>Schmid, 1978</td>
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<td></td>
<td>Hungary</td>
<td>26</td>
<td></td>
<td></td>
<td>12 m+10sm +4st</td>
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<td>Mészáros, Bartos, 1978</td>
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<tr>
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<td>26</td>
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<td>10m+16sm+</td>
<td>Ivanov, Madjanov, 1973</td>
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<td></td>
<td>Saratov Region Russia</td>
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<td>9q</td>
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<td>8m+14sm+4st</td>
<td>10m+12sm+4st+</td>
<td>Kaibeleva et al., 2004</td>
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<tr>
<td></td>
<td>Ukraine</td>
<td>26</td>
<td>9q</td>
<td>-</td>
<td>-</td>
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<td>8m+14sm+4st</td>
<td>Suryadnaya, 2003</td>
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<tr>
<td></td>
<td>China</td>
<td>26</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>14m+12sm</td>
<td></td>
<td>Gang Wei et al., 1992</td>
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</table>
might play an adaptive role. These variations can provide a genotypic adaptive advantage to changing environmental conditions faster than mutational processes (Prokofeva-Belgovska-ya, 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 *Pelophylax 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 (Spasić-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 *Pelophylax* complex. Thus, now in Russia and Ukraine *Pelophylax 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

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**Table 3** (continuation).

<table>
<thead>
<tr>
<th><em>R. macrocnemis,</em> form “camerani”</th>
<th>Armenia</th>
<th>26</th>
<th>10</th>
<th>-</th>
<th><em>1</em>(1, 2pi, 3q,4q 9p, 10q, 13q)</th>
<th>10</th>
<th>8m+12sm+6st</th>
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<td>Unknown</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>12m+8sm+6st</td>
<td>Popov, Dimitrov, 1999</td>
</tr>
<tr>
<td>Georgia</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>12m+10sm+4st</td>
<td>Ivanov, Madyanov, 1973</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><em>R. macrocnemis,</em> form “macrocnemis”</th>
<th>Kabardino-Balkaria; Karachai-Cherkess</th>
<th>26</th>
<th>-</th>
<th>-</th>
<th>-</th>
<th>-</th>
<th>10m+12sm+4st</th>
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<td>Russia Caucus</td>
<td></td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10m+16sm*</td>
<td>Birstein, 1984</td>
</tr>
</tbody>
</table>
In light of the analysis of chromosomal sets (Schmid, 1978; Mészáros, Bartos, 1978; Buc- 
ci et al., 1990; Spasić-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; Plöt-
ner 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 au-
thors (Ananjeva et al., 1998; Plötner, 2005) disagree on the frog’s distribution. The analysis 
of chromosomal complements of *P. ridi-
bundus* 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 distinc-
tions can be noted as intraspecific.

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

Comparison of chromosome sets of lake 
frogs in Armenia (*P. ridibundus*) and Europe 
(*R. fortis*) (Schmid, 1978; Mészáros, Bartos, 
1978; Bucci et al., 1990; Spasić-Bošković et 
al., 1999; Plötner, 2005; Plötner et al., 2008) 
reveals the differences in the number of chro-
mosomal pairs and C-banding patterns local-
ization, which can be explained as interspe-
ific.

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 Ara-
bia (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 reorga-
nizations (sm/st). Other pairs (7-th, 12-th) dif-
fer at the metacentric and submetacentric mor-
phological elements level. Taking into account 
the fact that the same species (*P. ridibundus*) 
habits 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. ridi-
bundus*) and in Western Turkey (*P. caralita-
enus*) 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).

<table>
<thead>
<tr>
<th>Sources</th>
<th>Chromosome pairs</th>
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<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Pelophylax ridibundus group</td>
<td></td>
</tr>
<tr>
<td>Armenia (8 populations)</td>
<td>m</td>
</tr>
<tr>
<td>Ukraine (Suryadnaya, 2003)</td>
<td>m</td>
</tr>
<tr>
<td>Saratov Region (Kaibeleva et al., 2004)</td>
<td>m</td>
</tr>
<tr>
<td>Kabardino-Balkaria Ukraine, Georgia (Ivanov, Madjanov, 1973)</td>
<td>m</td>
</tr>
<tr>
<td>Hungary (Mészáros, Bartos, 1978)</td>
<td>m</td>
</tr>
<tr>
<td>Cent. Europe (Schmid, 1978)</td>
<td>m</td>
</tr>
<tr>
<td>Yugoslavia (cur. Serbia), Macedonia (Spasić-Bošković et al., 1999)</td>
<td>m</td>
</tr>
<tr>
<td>Turkey (Beyschir) (Alpagut, Falakali, 1995)</td>
<td>m</td>
</tr>
<tr>
<td>Saudi Arabia (Al-Shehr, Al-Salech, 2005)</td>
<td>m</td>
</tr>
<tr>
<td>China (Gang Wei et al., 1992)</td>
<td>m</td>
</tr>
</tbody>
</table>

| Rana macrocnemis group | | | | | | | | | | | | | |
| Armenia (Ivanov, Madjanov, 1973) | m   | sm  | sm  | m   | sm  | m   | m   | st   | sm | *m | m   | sm | st  |
| Georgia (Ivanov, Madjanov, 1973) | m   | sm  | sm  | m   | m   | sm  | st  | st   | sm | m   | m   | sm  |
| Popov, Dimitrov, 1999 | m   | sm  | sm  | m   | sm  | m   | m   | st   | st | m   | m   | sm  |
| Russia, Caucasus (Birstein, 1984) | m   | sm  | sm  | m   | sm  | m   | sm  | *sm* | sm | *m | m   | sm  |
| Kabardino-Balkaria, Karachai-Cherkessia (Ivanov, Madjanov, 1973) | m   | m   | sm  | m   | m   | m   | m   | sm   | st | sm | m   | sm  |

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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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