The article presents data on genetic variability in populations of two brown frog species: the moor frog *Rana arvalis* and the siberian wood frog *R. amurensis*, in Western Siberia, Russia. Percentage of polymorphic ISSR-PCR-bands in *R. arvalis* was 63–93 %, in *R. amurensis* — 90 %, genetic diversity indices were 0.18–0.20 and 0.31, respectively. The high level of genetic variability in the siberian wood frog is contrary to its low population size, restricted distribution in the study area and the boundary position of the population. Some ISSR-PCR-bands were species-specific, they can be used for fast genotyping and further population genetic studies of the siberian wood and the moor frog in their areas of cohabitation.

**Materials and Methods**

Frogs were collected during July in 2011–2012 in surroundings of Tobolsk Biological Station of RAS “Missia” in Uvatsky area (58°20’N, 68°25’E), and near the Tyumen city (57°14’N, 65°26’E) in Tyumen region. A total number of 33 individuals of the siberian wood frog and 47 individuals of the moor frog were sampled.

We used the method ISSR-PCR (polymerase chain reaction of intersimple sequences repeats) to compare the genetic profiles of two frog species. ISSR-PCR method identifies polymorphisms between microsatellites sequence and has a high sensitivity for differentiation (Zietjiewicz et al., 1994). Total genomic DNA was extracted from cardiac muscle fixed in 70 % ethanol using the technique of alkaline lysis. Amplification was carried out using 25 μl of reaction mixture containing PCR buffer (0.01 M Tris-HCl, 0.05 M KCl, 0.1 % triton X-100), 4 mM MgCl2, 0.2 mM of each dNTPs, 1 μl of total DNA solution, 2.5 mM of primer and 0.2 unit/μL of Taq-polymerase (“Fermentas”), in the following mode: 94 °C — 7 min; then 94 °C — 30 sec, 52 (56) °C — 45 sec, 72 °C — 2 min (40 cycles); 72 °C — 7 min.
Six primers were used for ISSR-PCR (table 1). Analysis of ISSR-PCR-fragments was carried out on 2% agarose gel with using 1X Tris-EDTA-Borate buffer. The sizes of the fragments were determined using 100 bp DNA molecular weight markers (fragment length varies from 100 to 1000 bp, with step 100 bp). Electrophoretic gels were documented using VersaDoc system (Bio-Rad). Electrophoretic results were combined into binary matrices, where the presence of the band in gels was designated as “1” and was considered as a dominant allele; absence of the band was designated as “0” and considered as a recessive allele.

Standard population genetic characteristics — the percentage of polymorphic loci (P<sub>95%</sub>), observed number of alleles (n<sub>a</sub>), effective number of alleles (n<sub>e</sub>), Nei’s gene diversity (h<sub>Nei</sub>), Nei’s original measures of genetic identity (I<sub>Nei</sub>) and genetic distance (D<sub>Nei</sub>) (Nei, 1972), F-statistics (G<sub>ST</sub>), were computed using Popgen software (Yeh et al., 1999).

**RESULTS AND DISCUSSION**

Currently moor frog is very widespread in Western Siberia, it can be found in many habitats, including anthropogenically transformed landscapes. This species is 92–95% of the abundance of all species of amphibians that inhabit the taiga zone of Western Siberia. In contrast, the siberian wood frog has a narrow spread and uses a limited range of habitats, preferring floodplains. We found a population of siberian wood frog in Uvatsky area where it dwell sympatrically with the moor frog. This allowed us to obtain the first estimates of the genetic variability of the siberian wood frog in Western Siberia and to compare the genetic performance of two species.

We compared the genetic profiles of the siberian wood frog and the moor frog by method of ISSR-PCR. In total, it was studied 50 bands, from them only 32 were used for analysis (table 1, 2). Four (8%) bands were monomorphic and identical in both species (P2-5, P2-8, P4-5, P6-4). Some bands were typical only for R. arvalis or R. amurensis (Fig. 1–5). Species-specific bands for R. arvalis were P2-2, P3-2, for R. amurensis — P2-4, P2-6, P6-2. These bands can be used for species identification of the siberian wood and the moor frog in their areas of cohabitation.

The percentage of polymorphic bands of the siberian wood frog was 90%, in the moor frog from Uvatsky area — 93% (table 3). This index, as well as the observed number of alleles per locus were lower in the moor frog from Tyumen, the territory with high levels of anthropogenic

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**Table 1**

| Primer number | Primer code  | The composition of the primer | Primer annealing temperature t °C | Number of bands |
|---------------|--------------|-----------------------------|-----------------------------------|----------------|
| P1            | UBC-808      | (AG)<sub>1</sub>C           | 52                                | 11             |
| P2            | UBC-809      | (AG)<sub>1</sub>G           | 52                                | 10             |
| P3            | UBC-807      | (AG)<sub>1</sub>T           | 56                                | 12             |
| P4            | UBC-818      | (CA)<sub>1</sub>G           | 56                                | 7              |
| P6            | UBC-825      | (AC)<sub>1</sub>T           | 50                                | 6              |
| P7            | UBC-823      | (TC)<sub>1</sub>C           | 52                                | 4              |

**Table 2**

| Band number | R. arvalis | R. amurensis | Band number | R. arvalis | R. amurensis |
|-------------|------------|--------------|-------------|------------|--------------|
| P1-1        | +          | –            | P3-4        | +          | +            |
| P1-2        | +          | +            | P3-5        | +          | +            |
| P1-3        | +          | +            | P3-6        | +          | +            |
| P1-4        | +          | +            | P4-1        | –          | +            |
| P2-1        | –          | +            | P4-2        | –          | +            |
| P2-2        | –          | +            | P4-3        | –          | +            |
| P2-3        | +          | +            | P4-4        | –          | +            |
| P2-4        | –          | +            | P4-5        | +          | +            |
| P2-5        | +          | +            | P4-6        | +          | +            |
| P2-6        | –          | +            | P6-1        | +          | –            |
| P2-7        | –          | +            | P6-2        | –          | +            |
| P2-8        | +          | +            | P6-3        | –          | +            |
| P2-9        | +          | +            | P6-4        | +          | +            |
| P3-1        | +          | –            | P6-5        | +          | +            |
| P3-2        | +          | –            | P7-1        | +          | –            |
| P3-3        | –          | +            | P7-2        | –          | +            |

**Fig. 1.** Elctrophoregram of ISSR-PCR-pattern in frogs of genus *Rana*, with primer P1: 1-3, 6-8 — *Rana arvalis*, 4, 5 — *Rana amurensis*, M — DNA ladder 100bp (2% agarose gel, ethidium bromide, negative)
transformation. Reducing of the number of alleles and measures of polymorphism are typical for frog populations dwelling anthropogenically transformed landscapes (Hitchings, Beebee 1997). It can be due to genetic drift in small isolated populations of amphibian populations in the fragmentation of habitats.

Measures of genetic diversity of the siberian wood frog population in Uvatsky area were higher, compared to the moor frog population (table 3). This result was unexpected because the first species is relatively rare and has limited distribution. It was shown, that the value of genetic polymorphism of frogs is determined by the effective population size and gene flow to a greater degree than by the climatic and geographical factors (Sjogren-Gulve, Berg, 1999). Therefore, we should expect that the genetic diversity of the siberian wood frog would be lower in the study area of Western Siberia, as in the northwestern periphery of the area. High level of genetic variability of studied population shows its value for conservation of the species biodiversity.

Nei’s genetic identity \( (I) \) among moor frog populations was 0.829, distance \( (D) \) — 0.187. These indices between species were 0.782 and 0.246, respectively. The \( G_{ST} \) value as the measures of genetic subdivision was 0.20, this means that 80% of the genetic variability is accounted for intrapopulation one.

Despite the fact that the siberian wood frog and moor frog use the same spawning pond, they do not hybridize with each other. We found no hybrid individuals among the

| Levels of genetic variability of frog’s populations according to ISSR data |
|---------------------------------------------------------------|
| **Summary of genic variation statistics** | R. arvalis (n = 47) | R. amurensis (n = 33) |
|--------------------------------------------|----------------|-------------------|
| The percentage of polymorphic loci | Tyumen (n = 19) | Uvatsky (n = 28) | Tyumen (n = 19) | Uvatsky (n = 28) |
| Observed number of alleles \( (na) \) | 63.3 | 93.3 | 90.0 |
| Effective number of alleles \( (ne) \) | 1.6 | 1.9 | 1.9 |
| Index of Nei’s gene diversity \( (h) \) | 0.202 | 0.176 | 0.311 |
specimens studied. Different periods of spawning, as well as a different number of chromosomes confirm the fact of interbreeding inability. The siberian wood frog and the moor frogs belong to different groups of brown frogs on the genome size (Litvinchuk et al., 2008). There are 24 chromosomes in moor frog and 26 chromosomes — in siberian wood frog (Kim et al., 2002). While there are hybridogeneous species complex in the green frogs (Rana esculenta complex) (Lada et al., 1995), high level of genetic differentiation of species (Kim et al., 2002; Che et al., 2007), subspecies (Song et al., 2006; Litvinchuk et al., 2008) and populations (Palo et al., 2004; Zhang et al., 2010) is observed in brown frogs.

These two species differ not only genetically, but ecologically too. They differ in demographic characteristics and require different conservation strategies (Ishchenko, 1996). Siberian wood frog and moor frog had different sex-age composition of populations and population dynamics. There was an almost equal sex ratio in the population of the moor frog, where juveniles were 63 %. Proportion of males in a population of the siberian wood frog was three times more than females; most of them (82 %) were adults. The siberian wood frog population size was greater in 2011, but the moor frog population was more numerous than siberian wood frog in 2012. These differences were due to different seasonal dynamics, and can testify to the interspecies competition within the same biotope.

CONCLUSION

Level of genetic variability of Western Siberian moor frog populations is high, not lower, than in populations of Europe. Siberian wood frog also has a high level of genetic variation, which is contrary to its low population size, restricted distribution in the study area and the boundary position of the population. High level of genetic differentiation as well as reproductive isolation of the two studied frog species, even when they use the same spawning pond were detected. Some ISSR-PCR-bands were typical only for R. arvalis or R. amurenensis. These species-specific bands can be used for fast genotyping and further population genetic studies of the siberian wood and the moor frog in their areas of cohabitation.

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REFERENCES (TRANSLITERATED)

1. Abramson N. I., Rodchenkova E. N., Kostygov A. Y. (2009) Genetic variation and phylogeography of the bank vole (Clethrionomys glareolus, Arvicolinae, Rodentia) in Russia with special reference to the in-
trogression of the mtDNA of a closely related species, red-backed vole (Cit. rutilus). Rus. J. Genet. V: 5: P. 533–545.

2. Babik W., Branicki W., Sandera M., Litvinchuk S., Borkin L. J., Irwin J. T., Rafinski J. (2004) Mitochondrial phylogeography of the moor frog, Rana arvalis. Mol. Ecol. V: 13: P. 1469–1480.

3. Bender W., Pierre S., Hognes D. S. (1983) Chromosomal walking and jumping to isolate DNA from Ace and rosy loci of bithorax complex in Drosophila melanogaster. J. Mol. Biol. V: 168: P. 17–33

4. Che J., Pang J., Zhao E. M., Matsui M., Zhang Y. P. (2007) Phylogenetic relationships of the Chinese brown frogs (genus Rana) inferred from partial mitochondrial 12S and 16S rRNA gene sequences. Zoolog. Sci. V: 24(1): P. 71–80.

5. Hitchings S. P., Beebee T. J. C. (1997) Genetic substructuring as a result of barriers to gene flow in urban Rana temporaria (common frog) populations: implications for biodiversity conservation. Heredity. V: 79: P. 117–127.

6. Ishchenko V. G. (1996) Problems of demography and declining populations of some euroasiatic brown frogs. Russ. J. Herpetol. V: 3 (2): P. 143–151.

7. Kim J. B., Min M. S., Yang S. Y., Matsui M. (2002) Genetic relationships among Korean brown frog species (Anura, Ranidae), with special reference to evolutionary divergences between two allied species Rana dybowski and R. hurowensis. Zoolog. Sci. V: 19(3): P. 369–382.

8. Knopp T., Merilä J. (2009) Microsatellite variation and population structure of the moor frog (Rana arvalis) in Scandinavia. Mol. Ecol. V: 18 (14): P. 2996–3005.

9. Lada G. A., Borkin L. J., Vinogradov A. E. (1995) Distribution, population systems and reproductive behavior of green frogs (hybridogenetic Rana esculenta) in the Central Chernozem Territory of Russia. Russ. J. Herpetol. V: 2 (1): P. 46–57.

10. Litvinchuk S. N., Borkin L. J., Rosanov J. M. (2008) Genome size variation in Rana arvalis and some related brown frog species, including taxonomic comments on the validity of the R. arvalis subspecies. Z. Feldherpetol. V: 3: P. 95–112.

11. Nei M. (1972) The genetic distance between populations. Amer. Natur. V: 106: P. 283–291.

12. Palo J. U., Schmeller D. S., Laurila A., Primmer C. R., Kuzmin S. L., Merilä J. (2004) High degree of population subdivision in a widespread amphibian. Mol. Ecol. V: 13: P. 2631–2644.

13. Rafinski J., Babik W. (2000) Genetic differentiation among northern and southern populations of the moor frog Rana arvalis Nilsson in central Europe. Heredity. V: 84: P. 610–618.

14. Roček Z., Šandera M. (2008) Distribution of Rana arvalis in Europe: a historical perspective. Z. Feldherpetol. V: 13: P. 135–150.
15. Shapovalov S. I., Zhigileva O. N. (2002) Spatial variability of morphological and biochemical characters of Moor frog (\textit{Rana arvalis}) from three research locations. Contemporary Problems of Ecology. V. 6: P. 729–734.

16. Sjogren-Gulve P., Berg L. M. (1999) Allozyme variation as a demographic predictor at high latitudes: the Moor frog and the pool frog at 60 degrees N. Hereditas. V. 130 (3): P. 317–323.

17. Song J. Y., Matsui M., Chung K. H., Oh H. S., Zhao W. (2006) Distinct specific status of the Korean brown frog, \textit{Rana amurensis coreana} (Amphibia: Ranidae). Zool. Sci. V. 23 (2): P. 219–224.

18. Tanaka-Ueno T., Matsui M., Sato T., Takenaka S., Takenaka O. (1998) Local population differentiation and phylogenetic relationships of Russian brown frog, \textit{Rana amurensis} inferred by mitochondrial cytochrome \textit{b} gene sequences (Amphibia, Ranidae). Jap. J. Herpetol. V. 17 (3): P. 91–97.

19. Veith M., Kosuch J., Vences M. (2003) Climatic oscillations triggered post-Messinian speciation of Western Palearctic brown frogs (Amphibia, Ranidae). Mol. Phylogenet. Evol. V. 26: P. 310–327.

20. Yeh F. C., Yang R., Boyle T. (1999) POPGENE. Version 1.31. University of Alberta and Centre for International Forestry Research. Cited 24.01.2015. URL: http://www.ualberta.ca/~fyeh/popgene_download.html.

21. Zhang M., Jia X., Ma Y., Ma J. (2010) Genetic diversity and differentiation of the Dybowski’s frog (\textit{Rana dybowskii}) in Northeast China. J. Forest. Res. V. 21 (2): P. 239–245.

22. Zhigileva O.N., Kirina I.Y., Burakova A.V. (2014a) Genetic diversity and differentiation of the Moor frog (\textit{Rana arvalis}) in Western Siberia. Herpetology Notes. V. 7: P. 569–574.

23. Zhigileva O.N., Politov D.V., Golovacheva I.M., Petrovicheva S.V. (2014b) Genetic variability of sable \textit{Martes zibellina} L., pine marten \textit{M. martes} L. and their hybrids in Western Siberia: polymorphism of proteins and DNA. Rus. J. Genetics. V. 50 (5): P. 508–517.

24. Zietjewicz E., Raalshki A., Labuda D. (1994) Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics. V. 20: P. 176–183.

ПЕРВЫЕ СВЕДЕНИЯ О ГЕНЕТИЧЕСКОЙ ИЗМЕНЧИВОСТИ СИБИРСКОЙ ЛЯГУШКИ \textit{RANA AMURENSIS} В ЗАПАДНОЙ СИБИРИ И ЕЕ ДИФФЕРЕНЦИАЦИИ ОТ ОСТРОМОРДОЙ ЛЯГУШКИ \textit{RANA ARVALIS}

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Резюме: Представлены данные о генетической изменчивости популяций двух видов бурых лягушек: остромордой \textit{Rana arvalis} и сибирской \textit{Rana amurensis} в Западной Сибири. Доля ISSR-PCR-бэндов у \textit{R. arvalis} составила 63,3–93,3 %, у \textit{R. amurensis} — 90 %, индексы генетического разнообразия — 0,18–0,20 и 0,31 соответственно. Высокие показатели генетической изменчивости сибирской лягушки противоречат ее низкой численности, ограниченному распространению на исследуемой территории и краевому положению популяции. Некоторые ISSR-PCR-фрагменты были видоспецифичными, они могут быть использованы для быстрого генотипирования и дальнейших популяционно-генетических исследований сибирской и остромордой лягушек в местах их совместного обитания.

Ключевые слова: \textit{Rana amurensis}; \textit{Rana arvalis}; полиморфизм; ISSR-маркеры; совместное обитание; генетическая дифференциация; Западная Сибирь.

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