

# Species composition of green frogs (*Pelophylax Esculentus Complex*) of the Belgorod agglomeration based on DNA markers

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**Abstract.** On the basis of molecular genetic analysis of the intron-1 of the nuclear serum albumin gene (SAI-1) were identified 177 individuals of *Pelophylax esculentus* complex of 9 localities Belgorod. Two types of population systems R and RE were identified. Pure populations of L-type, E-type and LE-type as well as *P. lessonae* individuals were not identified.

## 1 Introduction

The European green frogs complex (*Pelophylax esculentus* complex) includes three species: *Pelophylax ridibundus* (Pallas, 1771), *P. lessonae* (Camerano, 1882) and *P. esculentus* (Linnaeus, 1758) [1]. According to studies by a number of authors, hybrid frogs are distributed almost all over Europe; this fact may indicate their "evolutionary success". Hybrids and their parents often live together and form common breeding groups, despite differences in their lifestyle [2].

Accurate identification of species of the European green frogs complex is difficult, and hybrid species often erase the "boundaries" of parent species, but it is this necessary to solve a number of problems: ecological monitoring, study of species introduction and invasion and to develop a strategy for their conservation [3, 4]. More precise molecular genetic methods should be used to reliably identify the species belonging to *Pelophylax esculentus* complex [2, 3].

The aim of the study is a reliable species identification of the European green frogs *Pelophylax esculentus* complex in the Belgorod and its surroundings.

## 2 Experimental

The material was collected during the summer field season 2018-19. In total 177 individuals from 9 localities of Belgorod region were studied by molecular genetic method: 1. «Severskiy Donec» 50.591/36.608 (n=14); 2. «Vezelka» 50.601/36.556 (n=17); 3. «Oskochnoe» 50.647/36.557 (n=20); 4. «Jachnevskiy» 50.639/ 36.579 (n=16); 5. «Dubovoe» 50.533/ 36.583 (n=17); 6. «Ust'e» 50.530/ 36.649 (n=26); 7. «Maiskiy» 50.515/ 36.462 (n=19); 8. «Severnyi» 50.733 36.487 (n=24); 9. «Shopino» 50.705/36.611 (n=24).

Molecular-genetic analysis was carried out Center of genomic selection of «Belgorod State National Research University».

The first phalanges of the hind leg were used as a tissue sample. To isolate genomic DNA, a set of DNA Extran-2 (Sintol) was used, according to the protocol proposed by the company. The obtained DNA solution was stored at -20°C.

Determine hybrids and cryptic forms, the nuclear serum albumin gene (SAI-1) were used according to the developed PCR test of the *Pelophylax esculentus* complex identification system proposed by Ivanov A. Yu. and Ermakov O. A. [5].

DNA variability was analyzed using polymerase chain reaction method. Samples were prepared per tube before amplification as follows: 2.5x Reaction mixture (2.5x PCR buffer B (KCl, TrisHCl (pH 8.8), 6.25 mM MgCl<sub>2</sub>), SynTaq DNA polymerase, deoxynucleoside triphosphates, glycerol, Tween 20 - 8 µl; MgCl<sub>2</sub> 25 mM - 0.5 µl; deionized water - 9.3 µl, mix of primers 0.1 µl (SA1F-Pu: CCATACAAATGTGCTAAGTAGGTT; SA1R-Pr: GATACAAATGATACATTCCACCT; SA1R-Pl: TACCGTACCGATATTTGTATGC; SA1R-Pb: TTGTTCCCTATACTAAGGTCAC).

PCR amplification was performed in the DNA amplifier Veriti (Thermo FS).

PCR amplification involved an initial cycle of denaturation at 95 °C for 5 min and 35 subsequent cycles of 94 °C for 30 s, 60 °C for 60 s, and 72 °C for 60 s, followed by a final extension step at 72 °C for 10 min.

After PCR, the amplification products were analyzed with electrophoresis in a 4% agarose gel followed by ethidium bromide staining and UV light imaging.

## 3 Results and discussion

Green frogs can form single-species "pure" (R, E and L) and mixed (RE, LE and REL) types of population systems. According to our study, 2 types of population systems - R and R-E types - have been identified in the Belgorod (Table 1).

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**Table 1.** Green frogs genotypes

Population	n	RR	BB	LL	BR	BL	LR
«Severskiy Donec»	14	3	4	0	4	2	1
«Vezelka»	17	5	7	0	5	0	0
«Oskochnoe»	20	2	9	0	3	4	2
«Jachnevskiy»	16	3	4	0	6	2	1
«Dubovoe»	17	0	0	0	1	11	5
«Ust'e»	26	1	2	0	4	12	7
«Maiskiy»	19	3	3	0	8	3	2
«Severnyi»	24	4	4	0	9	6	1
«Shopino»	24	1	7	0	8	4	4

Note: n - number of individuals; R - alleles of "western" form of marsh frog, B - alleles of "eastern" form marsh frog, L - alleles of pool frog.

The performed molecular genetic analysis revealed genetic heterogeneity of the green frog complex. Thus, on the urbanized territory of the Belgorod region, *P. cf. bedriagae* (BB) and its hybrids (BR, BL) dominate in water bodies, being one of the invasive representatives of amphibians, has a negative impact on local batrochofauna [6].

Our studies confirm that *P. cf. bedriagae* is confined to urbanized landscapes. Pure populations of L-type, E-type, and LE-type, as well as *P. lessonae* individuals, were not detected.

The data obtained should be taken into account in ecological studies of the region's batrochofauna, since *P. cf. bedriagae* has a higher resistance to anthropogenic press than other representatives of the *Pelophylax esculentus* complex [7,8,9].

## 4 Conclusion

As a result of the study, 2 species of green frogs complex were identified in the Belgorod agglomeration - *Pelophylax ridibundus* and *P. esculentus*. The study of nuclear DNA showed that the lake frog has alleles of two cryptic forms - "western" (*P. ridibundus*) and "eastern" (*P. cf. bedriagae*). It was found that both forms of the lake frog participate in the formation of hybrid individuals (*P. esculentus*).

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