SHORT COMMUNICATIONS
КРАТКИЕ СООБЩЕНИЯ

THE FIRST RECORD OF NATURAL TRANSFER OF MITOCHONDRIAL DNA FROM *PELOPHYLAX* CF. *BEDRIAGAE* INTO *P. LESSONAE* (AMPHIBIA, ANURA)

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The unidirectional natural transfer of mitochondrial (mt) DNA from *Pelophylax lessonae* into *P. ridibundus* is a common phenomenon in central Europe. Cases of mtDNA exchange between *P. lessonae* and other non-clonal species of the genus *Pelophylax* have been unknown so far. In this paper, we describe the first case of mtDNA transfer from *P*. cf. *bedriagae* into *P. lessonae*, which was found in National Park «Smolny», Republic of Mordovia, Russia.

Key words: European water frogs, hybridisation, hybridogenesis, interspecies introgression

Introduction

There are three species of western Palearctic water frogs of the *Pelophylax esculentus* complex inhabiting waterbodies in the East-European Plain (Hoffmann et al., 2015): the marsh frog, *Pelophylax ridibundus* (Pallas, 1771), pool frog, *P. lessonae* (Camerano, 1882) and a taxon of hybrid origin, the edible frog, *P. esculentus* (Linnaeus, 1758). Additionally, the Anatolian marsh frog (*P.* cf. *bedriagae*) has recently been revealed in some regions of Eastern Europe (Ermakov et al., 2014; Svinin et al., 2015; Zamaletdinov et al., 2015). The processes of hybridisation and hemiclonal reproduction in water frogs can lead to widespread introgression of genes in various parts of their ranges (Spolsky & Uzzell, 1984; Plötner et al., 2008; Doležálková et al., 2016; Morozov-Leonov, 2017; Čavlović et al., 2018). The unidirectional transfer of mitochondrial (mt) DNA from *P. lessonae* to *P. ridibundus* was previously found in Poland (Spolsky & Uzzell, 1984). Of 37 *P. ridibundus* specimens studied, 41% had mtDNA of the pool frog. In another study, 407 specimens of *P. ridibundus* from an area north of 48°N latitude and between 8°E and 22°E longitude were analysed (Plötner et al., 2008). Among them, 34% of individuals bear mtDNA of *P. lessonae*. According to the authors, such introgression may have occurred via the hybridogenetic *P. esculentus*. It is important to note the theoretically possible reverse variant of mtDNA introgression (i.e. from the marsh frog to the pool frog), but this has not yet been found.

Material and Methods

In June and August 2018, four individuals from two water bodies in Kemlyanskoe forestry of National Park «Smolny» (Ichalki district, Republic of Mordovia, Russia) were collected.

Pond 1 is a nearly round waterbody (50 × 50 m, depth up to 2 m) which is located near the village Smolny (54.732194°N, 45.296111°E). The pond is surrounded by a scarce pine (*Pinus sylvestris* L.) forest with an admixture of birch (*Betula pendula* Roth). The bottom of the pond is sandy and silty with a dense aquatic vegetation including floating on the surface of the water. It is used as a resting place and for grazing of cattle (Fig. 1).

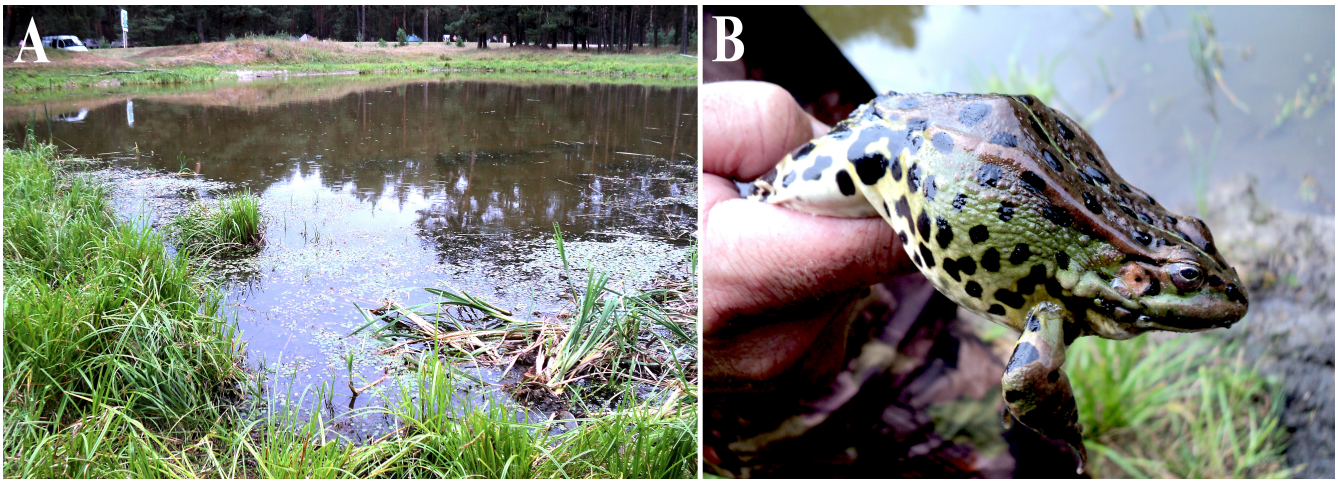

Fig. 1. A – pond 1 from Kemlyanskoe forestry of National Park «Smolny» (Russia); B – the edible frog (*Pelophylax esculentus*) from pond 1.

Pond 2 is an oval waterbody (24 × 180 m, depth up to 3 m; 54.721111°N, 45.284500°E) with a dense aquatic vegetation on the sides. It is located near the River Alatyr and has a sandy and silty bottom. The pond is used for cattle grazing.

The toe clips of the frogs were fixed in the field in 96% ethanol and were used as tissue samples. The DNA was extracted by the standard salt-extraction method (Aljanabi & Martinez, 1997) combined with lysis by proteinase K. The PCR reaction mixture (25 μL) contained 50–100 ng of DNA, 0.5 μM of each primer, 0.2 mM dNTPs, 1.5 mM MgCl2, 2.5 μL 10 × PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl), and 2 units of *Taq* polymerase (Thermo Scientific). The products of PCR and restriction hydrolysis were analysed by electrophoresis in 6% polyacrylamide gel (glass plate dimensions 8 × 10 cm) with further dying by ethidium bromide and UV visualisation. The molecular-weight size markers were the DNA kit of pBR322 plasmid processed with restrictase HpaII (pBR/*Hpa*II).

Identification of species was carried out using multiplex PCR based on species-specific differences in primary structure of the intron-1 of the nuclear serum albumin gene (*SA*I-1) (Ermakov et al., 2019). The nuclear *SA*I-1 gene was amplified using the primers Pel-SA-F1 5′-TCC ATA CAA ATG TGC TAA GTA GGT T-3′ and Pel-SA-R2 5′-GAC GGT AAG GGG ACA TAA TTC A-3′ (Hauswaldt et al., 2012). The PCR product of *P. lessonae* (genotype LL) has about 300 bp length, while *P. ridibundus* (RR) about 850 bp length (Fig. 2). The edible frog (which has a hybrid origin) is characterised by the presence of two bands corresponding to both parental species (RL).

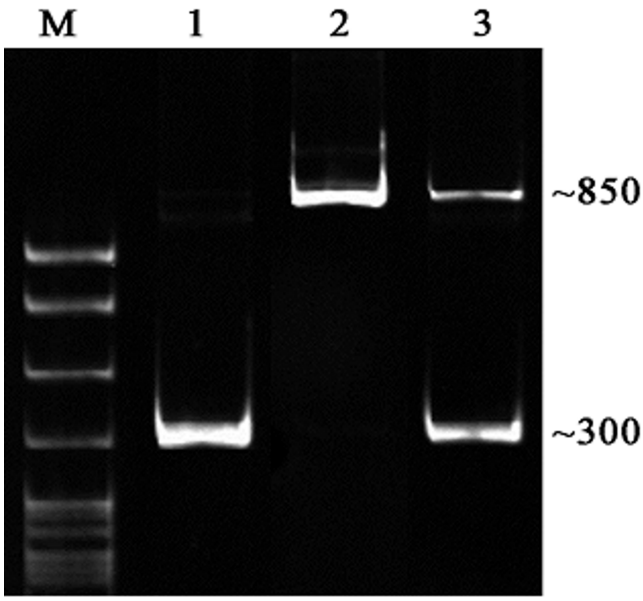

Fig. 2. Electrophoregram of PCR products of *SA*I-1 of *Pelophylax lessonae* (lane 1), *P. ridibundus* (2) and *P. esculentus* (3). M is a marker of molecular length. Length of products (in bp) is shown on the right side of the electrophoregram.

The mtDNA *ND*2 gene (1038 bp) was amplified with the universal primer ND2L1 5′-AAG CTT TTG GGC CCA TAC CCC-3′ (Meyer, 1993) and developed specific primer ND2H1 5′-GCA AGT CCT ACA GAA ACT GAA G-3′. The following amplification conditions were used: initial denaturation for 1 min at 95°C, followed by 32 cycles of 94°C for 30 s, 60°C for the *ND*2 and 53°C for the *SA*I-1 pair of primers for 30 s, 72°C for 60 s, and final extension for 5 min at 72°C.

Additionally, for an individual of the pool frog the primary sequences of both *ND*2 (1038 bp) and *SA*I-1 (262 bp) genes were studied. Sequencing was performed on an ABI 3500 automatic sequencer (Applied Biosystems), using the BigDye®Terminator

3.1 (Applied Biosystems) kit and the same primers that were used for amplification.

Results and Discussion

During the five years of our study on frogs, 135 individuals of water frogs from 15 localities in various regions of Central Russia were analysed by the use of multiplex PCR (Svinin et al., 2015; Zamaletdinov et al., 2015; Ivanov et al., 2016; Fayzulin et al., 2018). A natural introgression of mtDNA in any non-clonal species of water frogs has never been observed. We found only one record in National Park «Smolny». Of two individuals collected in pond 1, one was *P. lessonae* and the other was *P. esculentus*. Surprisingly, both these individuals carried mtDNA of *P.* cf. *bedriagae* (genotypes B-LL and B-RL respectively). In pond 2, only marsh frog individuals were observed. Both individuals collected here were characterised by nuclear (n) DNA of *P. ridibundus*, but mtDNA of *P.* cf. *bedriagae* (B-RR). Such combination of mt and nDNA markers is common for marsh frogs from the Middle Volga River basin (Ermakov et al., 2014; Zamaletdinov et al., 2015; Fayzulin et al., 2018).

The *ND*2 haplotype of an individual of the pool frog collected in National Park «Smolny» was absolutely identical to such in *P.* cf. *bedriagae* (GenBank number GU812116) from Atyrau (Kazakhstan; Akin et al., 2010), as well as numerous specimens of the species from the Volga River basin (Astrakhan region, Volgograd region and Nizhny Novgorod region, Republic of Tatarstan, Republic of Mari El and Republic of Mordovia of Russia; our unpublished data). The *SA*I-1 sequence of the individual of *P. lessonae* was 100% the same as in individuals of the species (FN432384) from Germany and Penza region of Russia (Plötner et al., 2009; our unpublished data).

The observed unidirectional transfer could be explained by an introgression of mtDNA via the hybridogenetic edible frog. For example, 6% of *P. esculentus* individuals studied previously by us had mtDNA of *P.* cf. *bedriagae* (Ermakov et al., 2019). Gametes with the *P. lessonae* genome can be produced by edible frogs from R-E- and R-E-L-systems (Dedukh et al., 2015, 2017; Svinin et al., 2015; Biriuk et al., 2016). The cross of an edible frog female, which produces oocytes with the *P. lessonae* genome (or gametes of both parental species) and bears mtDNA of *P.* cf. *bedriagae*, with a male pool or edible frog, which produces sperm with the *P. lessonae* genome, will result into offspring of the pool frog with mtDNA of *P.* cf. *bedriagae*.

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ПЕРВАЯ НАХОДКА ЕСТЕСТВЕННОГО ПЕРЕНОСА МИТОХОНДРИАЛЬНОЙ ДНК ОТ *PELOPHYLAX* CF. *BEDRIAGAE* К *P. LESSONAE* (AMPHIBIA, ANURA)

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Однонаправленный естественный перенос митохондриальной ДНК от *Pelophylax lessonae* к *P. ridibundus* – обычный феномен в Центральной Европе. Другие случаи обмена мтДНК между *P. lessonae* и другими неклональными видами рода *Pelophylax* не были известны. В настоящей работе мы описываем первый случай переноса мтДНК от *P.* cf. *bedriagae* к *P. lessonae*, найденный в национальном парке «Смольный», Республика Мордовия, Россия.

Ключевые слова: гибридизация, гибридогенез, европейские зеленые лягушки, межвидовая интрогрессия