

# First Documented Records of *Pelophylax lessonae* (Camerano, 1882) (Amphibia: Ranidae) from Bulgaria

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**Abstract:** The *Pelophylax esculentus* complex consists of two valid species, the Pool Frog (*P. lessonae*) and the Marsh Frog (*P. ridibundus*), and their natural hybrid the Edible Frog (*P. kl. esculentus*). Until now, only the latter two taxa have had confirmed localities for Bulgaria – *Pelophylax ridibundus* is ubiquitous across the country and *P. kl. esculentus* is distributed along the Danube River. Here we present two localities along the Danube River in northern Bulgaria with confirmed presence of *P. lessonae*: the first one (Oreh Island) based on bioacoustics data and the other one (Lake Srebarna) based on external morphological characteristics of a single captured specimen.

**Key words:** green frogs, bioacoustics, morphometry, distribution

## Introduction

The frogs of the genus *Pelophylax* Fitzinger, 1843 are widespread across Europe and Asia, with few species occurring in North Africa. The genus is currently estimated to include 21 species (FROST 2016), although not all are universally acknowledged. The genus comprises several hybrid forms including three hybridogenetic taxa (reviewed by PLÖTNER 2005). The best studied of these is the *Pelophylax esculentus* complex, which includes three species: the Pool Frog *P. lessonae* (Camerano, 1882), the Marsh Frog *P. ridibundus* (Pallas, 1771) and their natural hybrid, the Edible frog *P. kl. esculentus* (Linnaeus, 1758). *Pelophylax ridibundus* is distributed across most of Europe and from Russia to Afghanistan, Pakistan and parts of China (PLÖTNER 2005). *Pelophylax lessonae* occurs from France and southern Italy in the west to the Samara Province in Russia in the east; the northernmost localities are in southern Norway and the southernmost – from the north-western shore of the Black Sea. The range of *Pelophylax kl. esculentus* overlaps almost completely with that of its parental species (PLÖTNER 2005).

The *P. esculentus* complex is a well-studied example of hybridization and polyploidization. *Pelophylax kl. esculentus* reproduces hybridogenetically and forms three different genetic types: diploid individuals with one *lessonae* (L) and one *ridibundus* (R) genome (genotype LR) and triploid individuals that possess either two L genomes and one R genome (genotype LLR) or two R genomes and one L genome (genotype LRR) (HOFFMANN et al. 2015). Although very rare, there are also cases of viable tetraploid individuals (LLRR) (e.g. ARIOLI et al. 2010, JAKOB et al. 2010).

When determining the taxonomic affiliations of species from the *P. esculentus* complex using external morphological traits, the relative lengths of the metatarsal tubercle (callus internus) and the hind legs are of vital importance; thigh coloration is also significant (BERGER 1973). Although the values of these traits are partially overlapping between the separate taxa, they are accepted as key for species determination in almost every guide on European herpetofauna (e.g. BANNIKOV et al. 1977, ENGELMANN

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et al. 1985, ARNOLD & OVENDEN 2002, STOJANOV et al. 2011, SPEYBROECK et al. 2016).

Anuran mating calls are generally accepted as having taxonomic value, since they can often be used to distinguish between conspecific specimen and individuals from different species (WELLS 2010). Studies on the qualitative and quantitative characteristics of *P. lessonae* mating call date as far back as the late 70's of the 20<sup>th</sup> century (SCHNEIDER et al. 1979). The bioacoustic method has been used in previous studies on its distribution and taxonomic status (SCHNEIDER & JOERMANN 1988, HAXHIU 1994, SCHNEIDER & HAXHIU 1994, SINSCH & SCHNEIDER 1996).

In Bulgaria, studies on *Pelophylax esculentus* complex are scarce. It is thought that the Marsh Frog has a ubiquitous distribution, while the Edible Frog has only been found along the Danube River bank and in Lake Durankulak (BESCHKOV 1965, BATSHVAROV & POPOV 1973, BESHKOV & NANEV 2002, STOJANOV et al. 2011). The possible presence of the Pool Frog has been discussed by BISERKOV et al. (2007), STOJANOV et al. (2011) and, in detail, by BISERKOV & NAUMOV (2012) suggesting that some of the specimens from Lake Srebarna described by BESCHKOV (1965) are probably *P. lessonae*. According to TZANKOV & POPGEORGIEV (2014), *P. lessonae* is currently found in the country; they mention Lake Srebarna as a locality and suggest the species is distributed in northern and central Bulgaria, without providing any additional information.

The present article provides the first documented data proving the presence of *Pelophylax lessonae* at two localities in Bulgaria.

## Materials and Methods

The studied material is from two localities along the Bulgarian sector of the Danube River (Fig. 1):

1. The Oreh (Esperanto) Island (N43.7225°, E24.0175°) near the town of Oryahovo. We recorded a total of 39 calls of *P. lessonae* during the frogs' breeding season (April-June) in 2012-2013 in the course of two expeditions. Calls emitted by two or more animals simultaneously or distorted by noise were discarded and not used in the analyses. Air and water temperatures were measured using a digital thermometer with an accuracy of 0.1°C. For the first visit, they were 19.4°C and 21.3°C, and for the second – 24.5°C and 27.2°C (for air and water, respectively). The vocal activity was recorded using an Olympus LS-5 linear PCM recorder and an Olympus ME-31 shotgun microphone at a distance of up to 10 m from the calling frog. We recorded the mating calls in a WAV-PCM mode with sampling frequency of 44.1

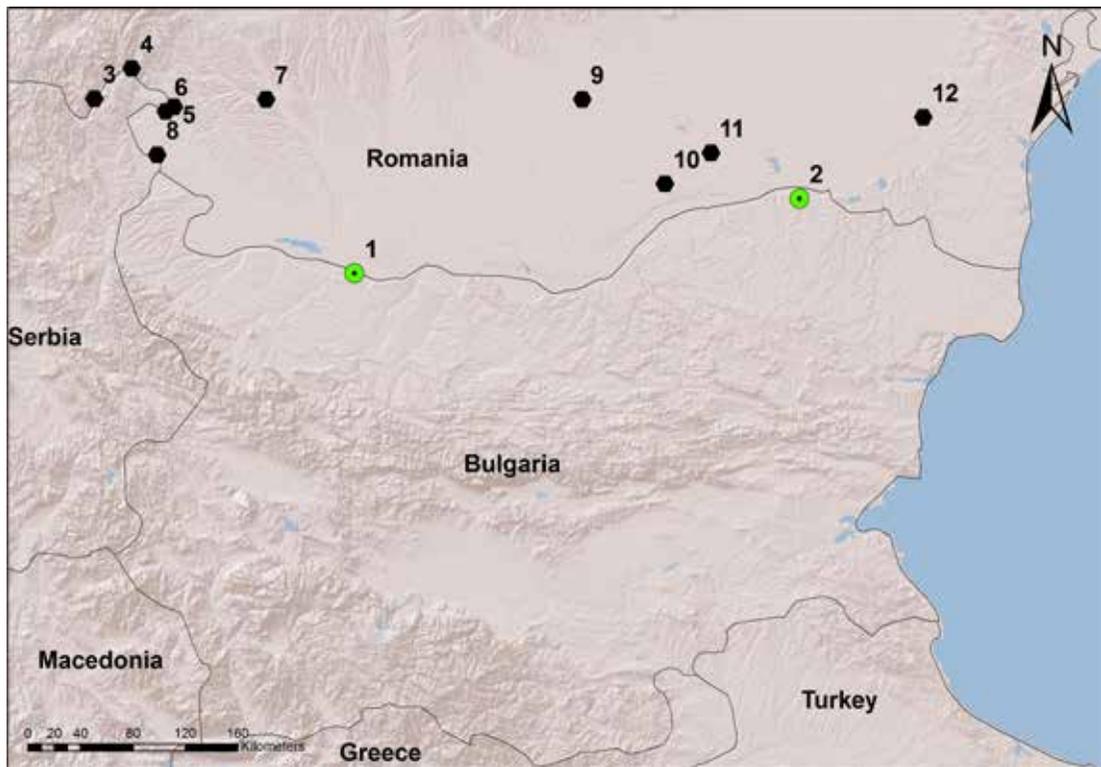
kHz, 20 - 21.000 Hz and 24 bit resolution. The recordings were processed with Soundruler v. 0.9.6.0. (GRIDI-PAPP et al. 2007) and AviSoft-SASLab Pro v. 5.2 (AVISOFT BIOACOUSTICS 2017). Soundruler was also used to visualise the calls via oscillograms and spectrograms. We analysed the calls in respect to call duration, intercall interval, number of pulses, pulse duration, interpulse interval, fundamental frequency and relative amplitude. All durations were measured in seconds (s) and frequency was measured in hertz (Hz). All parameters are presented with descriptive statistics: mean, standard deviation and range (min-max values). We used a Kruskal-Wallis test for comparison between the two sets of calls (recorded at different temperatures).

2. The south-western shore of Lake Srebarna (N44.0898°, E27.0698°), Silistra District. In June 2016, we collected a specimen with external morphological characteristics similar to those of the Pool Frog or the Edible Frog. The specimen was preserved in 70% ethanol and the following measurements were taken using an electronic calliper: snout-vent length (L.), head width (Lt.c.), distance between nostril and frontal end of the eye (D.n.o.), length of the tibia (T.), length of the first toe of rear leg (D.p.) and length of the inner metatarsal tubercle (C.int.).

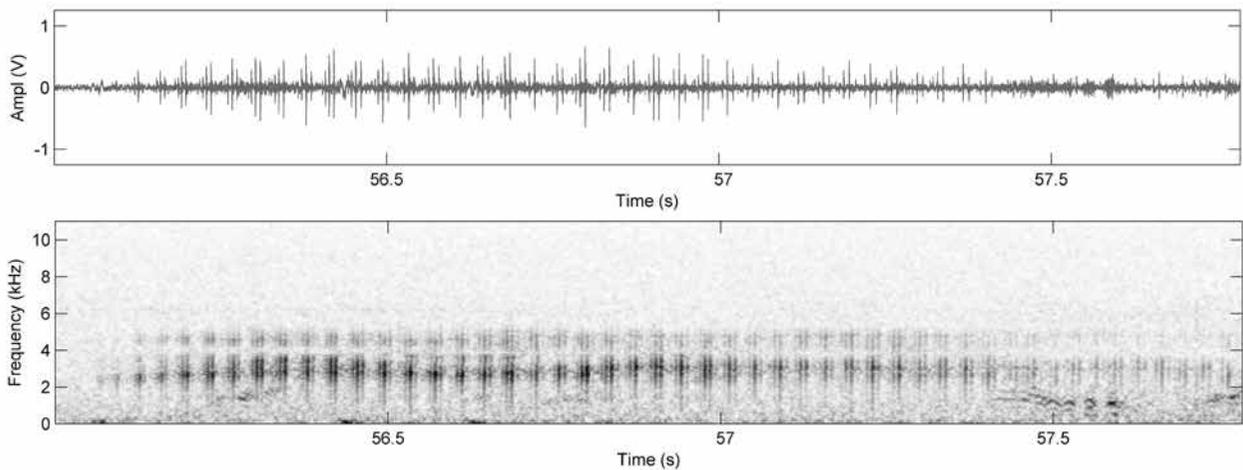
## Results and Discussion

The first locality of *P. lessonae* in Bulgaria is the Oreh Island (Fig. 1), where we recorded mating calls of the species. The call had a clear harmonic structure, with mean dominant frequency of  $2030 \pm 868.43$  Hz (63 - 3438 Hz). The second of three harmonics was interpreted as the fundamental frequency and its relative amplitude was  $16.67 \pm 6.27$  (14.26 - 19.01). The total call duration was  $1.40 \pm 0.44$  s (0.67 - 2.37 s; Fig. 2). Calls were normally emitted in short bursts of 4 - 6 in a group, with intercall interval of  $0.4 \pm 0.2$  s (0.2 - 0.6 s; Fig. 3). The average number of pulses was  $82 \pm 47.94$  (31 - 226), with pulse duration of  $0.0008 \pm 0.0017$  s (0.0003 - 0.0824 s) and interpulse interval  $0.0130 \pm 0.0112$  s (0.0015 - 0.1817 s). The comparison between the two sets of calls (regarding temperature) revealed statistically significant difference in terms of pulse number ( $p < 0.01$ ) and call duration ( $p < 0.05$ ). Calls recorded in higher temperature contained more pulses ( $99.11 \pm 47.84$ ; 32 - 226) and had lower duration ( $1.30 \pm 0.45$  s; 0.67 - 2.37 s) than those recorded in lower temperature –  $43.67 \pm 13.57$  (31 - 77) and  $1.64 \pm 0.35$  s (1.00 - 2.37 s).

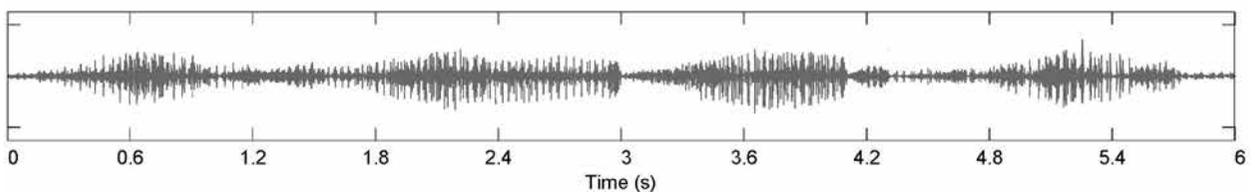
According to SINSCH & SCHNEIDER (1996), the mating call of topotypical *P. lessonae* is characterised by absence of intervals between the pulse groups, with



**Fig. 1.** Positions of the localities of *P. lessonae* in Bulgaria (1 and 2: the Oreh Island and Lake Srebarna, this study) and the nearest records from Romania: 3-7 – Plavișevița, Vodița Monastery, Hinova, Ostrovul Corbului and Arginești (COVACIU-MARCOV et al. 2008); 8-12 – 5x5 km UTM grid cells without locality descriptions (COGĂLNICEANU et al. 2013).



**Fig. 2.** Oscillogram and spectrogram of a *P. lessonae* call from the Oreh Island (pulses are clearly visible but do not form distinct pulse groups).



**Fig. 3.** A series of four calls, demonstrating the shorter intercall interval compared to call duration during the burst (recorded on the Oreh Island).

**Table 1.** Numerical values for five morphometric indices (for abbreviations see Materials and Methods) in the three taxa of *P. esculentus* complex (literature data) and the specimen from Lake Srebarna (present study).

	<b>D.p./C.int.</b>		
	<i>P. lessonae</i>	<i>P. kl. esculentus</i>	<i>P. ridibundus</i>
ENGELMANN et al. (1985)	< 2.1	2.0 – 2.8	> 2.5
GÜNTHER (1990)	< 2.1	2.0 – 2.5	> 2.3
PLÖTNER (2005)	1.3 – 1.7	1.7 – 2.9	2.9 – 3.9
KRIZMANIĆ (2008)	1.2 – 1.9	1.6 – 3.0	2.1 – 3.8
This study	1.73		
	<b>T./C.int.</b>		
	<i>P. lessonae</i>	<i>P. kl. esculentus</i>	<i>P. ridibundus</i>
ENGELMANN et al. (1985)	< 7.0	6.5 – 9.0	> 8.5
GÜNTHER (1990)	< 7.0	7.0 – 8.5	> 8.0
PLÖTNER (2005)	< 7.0	6.5 – 9.4	> 8.0
KRIZMANIĆ (2008)	4.3 – 7.7	6.4 – 11.8	7.6 – 15.7
This study	6.12		
	<b>L./T.</b>		
	<i>P. lessonae</i>	<i>P. kl. esculentus</i>	<i>P. ridibundus</i>
ENGELMANN et al. (1985)	n/a	> 2.0	< 2.0
GÜNTHER (1990)	> 2.2	> 2.0	< 2.0
KRIZMANIĆ (2008)	1.9 – 3.0	1.7 – 2.5	0.9 – 2.2
This study	2.15		
	<b>Lt.c./C.int.</b>		
	<i>P. lessonae</i>	<i>P. kl. esculentus</i>	<i>P. ridibundus</i>
PLÖTNER (2005)	3.5 – 5.3	4.5 – 7.2	6.3 – 9.3
This study	4.44		
	<b>D.n.o./C.int.</b>		
	<i>P. lessonae</i>	<i>P. kl. esculentus</i>	<i>P. ridibundus</i>
PLÖTNER (2005)	2.2 – 2.8	2.5 – 3.8	3.1 – 4.7
This study	2.13		

low number of pulses in each group. This description presents a near perfect match for the mating calls we recorded in the Oreh Island: while pulses were clearly visible, they did not form pulse groups and the entire call had a more uniform structure than those of other syntopic water frogs. The call of *P. ridibundus* has a very pronounced group structure, with 5 - 7 pulse groups per call and 20 pulses per pulse group (SCHNEIDER & SINSCH 1992). While the call of *P. kl. esculentus* resembles that of *P. lessonae* (see LODE 2001), generally it is shorter in duration, with less pulses per call (15–26) and more distinct pulse groups (SINSCH & SCHNEIDER 1996, ZAKS 2008). SINSCH & SCHNEIDER (1996) recorded a call duration for Italian *P. lessonae* of 1.40 s, with single calls consisting of about 120 pulses per call at 21°C. When studying *P. lessonae* populations from western and central France, LODE (2001) recorded mean call duration of 1.12±0.25 s with 33 pulses on average; all studied specimens were also genetically identified. According to ZAMFIRESCU (2004), mean call duration for a Romanian population of *P. lessonae* is

1.31 ± 0.27 s; he states that the calls are emitted in series of 4 - 8 bursts and “One call includes 30 - 40 pulses with similar relative amplitudes between the frequencies of 500 - 3000 Hz”. The author did not provide the temperature at which the recordings were made. In the work of ZAKS (2008) call duration is given as 790 ± 267.9 ms, with 33 ± 3.2 pulses at 24°C in a lake near the city of Penza, Russian federation. While different, these results are rather comparable to our acoustic data and we could hypothesise that the call characteristics of Balkan populations have intermediate values between German and Russian populations. ZAKS (2008) also provides data on mating calls from Germany: duration 1740.3 ± 585.1 ms, with pulse number of 39 ± 2.9; however, the author states that these recordings are from an audio CD “Heimische Froschlurche, Rufe zur Paarungszeit” and we think they have to be taken with caution. Considering this, the registered differences could easily be contributed to regional intraspecific variability and local environmental factors.

The second locality of *P. lessonae* in Bulgaria



**Fig. 4.** Photographs of the specimen from Lake Srebarna (above: callus internus photographed through a stereomicroscope under 6.5× magnification after the specimen was fixed in ethanol; below: photographs of the dorsal and ventral side of the specimen at the time of collection).

is Lake Srebarna. The single collected specimen had the following morphometric traits: L. = 47.00 mm, Lt.c. = 15.88 mm, D.n.o. = 7.60 mm, T. = 21.91 mm, D.p. = 6.19 mm, C.int. = 3.58 mm. Table 1 presents a comparison of calculated indices for our specimen and those given for the three taxa of the *Pelophylax esculentus* complex in some comprehensive publications (ENGELMANN et al. 1985, GÜNTHER 1990, and PLÖTNER 2005) as well as the study of KRIZMANIĆ (2008) on the Edible Frog in Serbia. According to the summarised index values, our specimen could be classified only as *P. lessonae*. The shape of callus internus resembles a semi-circle (Fig. 4), which is also typical of *P. lessonae* (see BERGER 1973, ENGELMANN et al. 1985, PLÖTNER 2005). Regarding the species-specific coloration traits (as given by PLÖTNER 2005), the Srebarna specimen (Fig. 4) can be described as follows: 1) dorsal side brown with few indistinctly outlined dark spots (typical mainly of *P. lessonae*); 2) ventral side white and marbled with pale, grey to grey-brown spots (typical mainly of *P. kl. esculentus*); 3) inner side of thighs with a

bright-yellow line, and the outer – with bright-yellow spots (typical mainly of *P. lessonae*).

Considering the presented comparisons, we conclude that the examined specimen from Lake Srebarna could be attributed to *P. lessonae*.

The nearest known localities of *P. lessonae* are on Romanian territory, at about 100-130 km north-west from the Oreh Island and 50-70 km north-west and west from Lake Srebarna, respectively (see Fig. 1). The Bulgarian localities are important for drawing the southern border of this species range. We hypothesise that *P. lessonae* probably also occurs in other localities along the Bulgarian bank of the Danube River but more targeted studies and application of wider range of methods for species determination are needed in order to clarify its distribution and the population structure of the *P. esculentus* complex in Bulgaria.

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