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# First bioacoustic and morphological data for the presence of *Pelophylax bedriagae* in Bulgaria

Simeon Lukanov [1], Georgi Popgeorgiev[2], Nikolay Tzankov[2]

[1] *Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Tzar Osvoboditel № 1, 1000 Sofia, Bulgaria.*

[2] *National Museum of Natural History, Bulgarian Academy of Sciences, blvd. Tzar Osvoboditel 1, 1000 Sofia, Bulgaria*

e mail: simeon_lukankov@abv.bg

**Abstract:** *Water frog mating calls from two localities were studied and analyzed. Recordings were made in the summer of 2010 at the Arkutino swamp near the town of Primorsko and at the Vurbitza River near the town of Momchilgrad. A total of 154 calls were analyzed and the results suggested the presence of both the Marsh frog (Pelophylax ridibundus) and the Levant frog (Pelophylax bedriagae) in both sites, with the former being more frequent in Vurbitza River, and the latter – in Arkutino. At Vurbitza, we also captured and measured 2 specimens, which morphological characteristics differed from P. ridibundus and matched those of P. bedriagae. These are the first localities for P. bedriagae in Bulgaria.*

**Keywords:** *distribution, Levant frog, locality, mating call, measurements.*

## Introduction

Until the 80's the Marsh frog, *Pelophylax ridibundus* (Pallas, 1771), was regarded as a species with a very broad distribution. Its range was thought to include most of Europe, Asia and the Near East, as well as parts of the Arab peninsula and the Nile valley [1]. Throughout the 80's and 90's bioacoustics studies, backed by allozyme analyses and – sometimes – morphological characters, began to change this picture. The existence of the Levant frog, *Pelophylax bedriagae*, as a separate species, was demonstrated by allozyme analysis in the late 80's [2] and later confirmed by electrophoretic studies [3] and differences in morphological characters [4]. Different bioacoustics studies on mating (advertisement) calls, mainly from the Mediterranean, proved the presence of *P. bedriagae* in many regions where previously *P. ridibundus* was thought to occur [5], [6], [7], [8], [9], [10].

*P. ridibundus* is distributed mainly in central and eastern Europe and the Balkans, while the taxonomic status of the Asian populations is yet unclear [11], [12]. The southern margin of distribution is insufficiently known because many new species are described from different geographic populations in these parts of its range [5], [13], [9]. Bioacoustic studies on frog populations from Greece, Bulgaria and Armenia have demonstrated the advertisement calls to be consistent with those from the type locality of the species – Atyrau, Kazakhstan [6], [14], [15], [1], [16], [17]. In Bulgaria, *P. ridibundus*


Corresponding author: simeon_lukanov@abv.bg
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is ubiquitous across the country, living in syntopy with *Pelophylax kurtmuelleri* (Gayda, 1940) to the South-West [18] and both the *Pelophylax* kl. *esculentus* (L., 1758) and *Pelophylax lessonae* (Camerano, 1882) along the Danube River [19]. Turkey was also considered part of *P. ridibundus* range, but in the late 80's bioacoustics study concluded that the populations from Izmir and Dalaman represented a different form [7], which later bioacoustics and morphometric studies identified as *P. bedriagae* and expanded its range to other parts of the country [9], [10]. As of today, *P. bedriagae* is also accepted to occur on the island of Rhodes and other North Aegean and Dodecanese islands in Greece, much of Cyprus, East Syria (with isolated populations in the North), Lebanon, Northeastern Jordan, much of Israel and Egypt [20]. For Bulgaria the species is mentioned by [19] as having a limited distribution in the southeastern areas, but the authors do not provide any additional information.
The present paper uses bioacoustics and morphometric data to demonstrate the first records of *P. bedriagae* for Bulgaria.

## Materials and Methods

All recordings, as well as the morphological measurements, were made in June and July of 2007 and 2010, and served as the basis on which [19] decided to include *P. bedriagae* in the Bulgarian batrachofauna. Here we provide a detailed analysis of the calls and present the morphometrics as well as the localities they were recorded from (Figure 1). 154 mating calls from two localities from southeast Bulgaria recorded and analysed. The first is Arkutino a swamp in the district of Burgas, 2.5 km to the northwest of the Ropotamo River mouth. It is approximately 400 m long and 200 m wide, with a depth of about 0.5 m; it is separated from the Black sea by a sand strip and has a low salinity of 0.2 ‰. At this location a total of 28 mating calls were recorded. The second locality is the Vurbitza River, near the town of Momchilgrad. Vurbitza is the longest and deepest tributary of Arda River, running for approximately 98 km, and is one of the main sources for drinking water in the region. It supports three large and numerous small dams that have important role in water level regulation and local irrigation. Here we recorded mating calls from two locations 3 km apart: Vurbitza 1 (100 calls) and Vurbitza 2 (26 calls). Morphometric measurements were taken from specimens at the Vurbitza 1 location.


Corresponding author: simeon_lukanov@abv.bg
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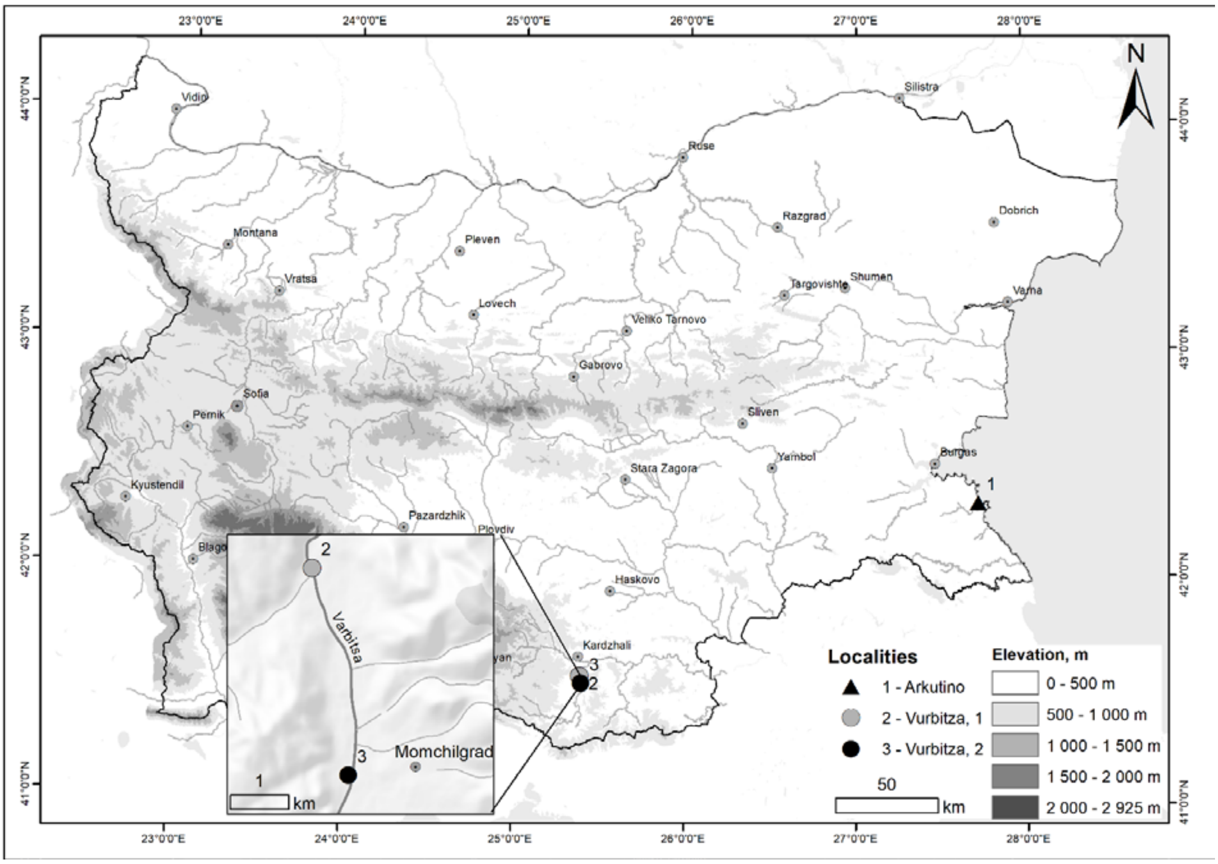

**Fig. 1.** A map with the localities for *P. bedriagae*.

Air (up to 0.5 m) and water temperatures of the localities were measured because they have great influence on amphibian activity. The temperatures were measured using a digital water-resistant thermometer with an accuracy of 0.1 of the degree. For Arkutino we measured 25.3˚C and 24.3 ˚C for air and water, respectively; the samples from Vurbitza were collected at two locations, about 5km apart, and the respective temperatures were 22.3 ˚C (19.8 ˚C) and 22.7 ˚C (21.7 ˚C).

Call recordings and measurements were taken once per day during the midday hours (14-16 h). The vocal activity was recorded using an Olympus LS-5 linear PCM recorder and an Olympus ME-31 shotgun microphone. 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 the computer program Soundruler V. 0.9.6.0. [21]. For the statistical analyses we used the following call parameters: energy between initial 10:50%, peak amplitude (Ener_10-50_Beg), energy between initial 50:90% peak amplitude (Ener_50-90_Beg), energy between final peak:90% amplitude (Ener_Peak-90_End), energy between final 90:50% peak amplitude (Ener_90-50_End), energy between final 50:10% peak amplitude (Ener_50-10_End), dominant frequency of the pulse (PulseDomFreq), fundamental frequency of the pulse (PulseFundFreq), minimum of dominant frequency in the pulse (PulseMinFreq), maximum of dominant frequency in the pulse (PulseMaxFreq), onset pulse dominant frequency (PulseOnFreq), offset pulse dominant frequency (PulseOffFreq), prop of duration to reach half frequency modulation (PulseHalfFM), tuning: peak frequency/bandwidth at 50% peak amplitude (Tuning-6dBSPL), tuning: peak frequency/bandwidth at 10% peak amplitude (Tuning-20dBSPL), call duration in seconds (Call dur.), pulse group duration (PG dur.), pulse group intervals (PG inter.), pulse group periods (PG per.) and pulse group number (PG Number).


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The following morphological measurements were taken using an electronic calliper: L. (snout-vent length), F. (femur length), T. (tibia length), D.p. (length of the first toe of rear leg), C.s. (metatarsal articulation length), C.int. (length of the inner metatarsal tubercle), C.int.2 (width of the inner metatarsal tubercle). The following proportions were also used: D.p./ C.int., T./ C.int., Ix, L./T., L./ C.int. and L./ D.p.

Data was checked for normality with a Shapiro-Wilk test and then we performed an analysis of variance (ANOVA) with two grouping variables: locality (A – Arkutino, V – first Vurbitza location and V2 – second Vurbitza location) and designated group (r – ridibundus and b – bedriagae), with post-hoc least significant difference tests at $p < 0.05$. A principal component analysis (PCA) was performed to determine which variables were more valuable for distinguishing between groups. A canonical discriminant analysis (CDA) was used to find the Mahalanobis distances between the groups. All analyses were carried out using the computer program Statistica v.7.0 [22].

## Results

Descriptive statistics of the calls from the three localities are presented on Table 1. ANOVA results demonstrated that call characteristics significantly differed across the designated groups ($p<0.05$). The post-hoc tests revealed differences between calls from Arkutino and both Vurbitza locations in terms of call and pulse group duration and interval; the Vurbitza locations differed between each other in parameters related to energy and frequency, but there were no differences in terms of PG number. Most of the sound energy was concentrated in the region between 0.50 and 3 kHz, which includes the dominant frequency band (1.91–3.0 kHz) (Table 1; Figure 2).

**Table 1.** Descriptive statistics of studied call parameters. Data is presented as Mean (Min-Max ± SD). Frequency is measured in kHz and duration in seconds. N = 28 (A), 102 (V1) and 22 (V2).

| Parameter | Locality | | |
|---|---|---|---|
| | Arkutino | Vurbitza 1 | Vurbitza 2 |
| **Ener_10-50_Beg** | 0.40 (0.001-2.33 ± 0.59) | 2.65 (0.35-5.87 ± 1.44) | 0.51 (0.09-1.38 ± 0.36) |
| **Ener_50-90_Beg** | 0.18 (0.06-1.02 ± 0.17) | 2.18 (0.07-5.87 ± 1.54) | 0.13 (0.03-0.51 ± 0.12) |
| **Ener_Peak-90_End** | 0.19 (0.05-0.40 ± 0.09) | 0.53 (0.06-2.16 ± 0.41) | 0.06 (0.01-0.13 ± 0.04) |
| **Ener_90-50_End** | 0.18 (0.06-0.87 ± 0.15) | 1.98 (0.06-6.23 ± 1.38) | 0.13 (0.03-0.34 ± 0.09) |
| **Ener_50-10_End** | 0.39 (0.05-1.60 ± 0.43) | 2.56 (0.23-6.63 ± 1.55) | 0.62 (0.12-1.59 ± 0.41) |
| **PulseDomFreq** | 2.27 (1.91-2.64 ± 0.23) | 2.33 (1.9-3.00 ± 0.25) | 2.62 (2.20-4.12 ± 0.40) |
| **PulseFundFreq** | 1.13 (0.96-1.32 ± 0.12) | 1.16 (0.95-1.5 ± 0.13) | 1.31 (1.10-2.06 ± 0.20) |
| **PulseMinFreq** | 2.24 (1.90-2.58 ± 0.22) | 2.27 (1.87-2.95 ± 0.25) | 2.58 (2.14-4.05 ± 0.40) |
| **PulseMaxFreq** | 2.29 (1.92-2.70 ± 0.24) | 2.38 (1.99-3.04 ± 0.25) | 2.67 (2.23-4.16 ± 0.41) |


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| | | | |
|---|---|---|---|
| **PulseOnFreq** | 2.27 (1.91-2.66 ± 0.24) | 2.32 (1.91-3.03 ± 0.25) | 2.63 (2.21-4.13 ± 0.41) |
| **PulseOffFreq** | 2.26 (1.91-2.62 ± 0.23) | 2.33 (1.95-2.95 ± 0.25) | 2.61 (2.17-4.09 ± 0.40) |
| **PulseHalfFM** | 0.87 (0.39-1.35 ± 0.25) | 0.48 (0.21-1.03 ± 0.13) | 0.67 (0.42-1.53 ± 0.25) |
| **Tuning-6dBSPL** | 25.84 (20.86-35.71 ± 4.50) | 30.29 (18.84-43.32 ± 4.31) | 33.81 (23.21-61.09 ± 7.91) |
| **Tuning-20dBSPL** | 4.48 (3.04-7.00 ± 0.92) | 6.41 (4.27-9.87 ± 1.45) | 6.66 (3.66-14.60 ± 2.24) |
| **CallDur** | 0.72 (0.50-0.90 ± 0.11) | 0.50 (0.30-0.70 ± 0.10) | 0.58 (0.40-0.80 ± 0.13) |
| **PG Dur** | 0.03 (0.02-0.04 ± 0.006) | 0.036 (0.03-0.04 ± 0.004) | 0.039 (0.3-0.4 ± 0.002) |
| **PG int** | 0.02 (0.015-0.035 ± 0.005) | 0.03 (0.025-0.035 ± 0.004) | 0.03 (0.025-0.04 ± 0.005) |
| **PG period** | 0.05 (0.04-0.07 ± 0.01) | 0.07 (0.06-0.08 ± 0.003) | 0.07 (0.065-0.080 ± 0.006) |
| **PG number** | 15.86 (8-20 ± 4.06) | 7.8 (5-11 ± 1.30) | 8.32 (6-13 ± 2.17) |

According to the PCA, the variables with higher factor weight were those related to frequency, call duration, PG interval and PG number. These were chosen for further processing with CDA. The first four axis explained 73% of the total intergroup variation (34%, 24%, 9% and 6% respectively).Despite the partial overlap, the three groups were still fairly well differentiated, with most overlapping occurring between the two Vurbitza locations (Figure 2). Most of the calls from Arkutino were consistent with those of *P. bedriagae*, while in Vurbitza the results were mixed, with *P. ridibundus* seemingly predominant in both locations and *P. bedriagae* calls more frequent in the second Vurbitza location. The CDA revealed a significant Mahalanobis distances between the three groups – 26.7 between Arkutino and Vurbitza 1, 29.5 between Arkutino and Vurbitza 2, and 14.2 between the two Vurbitza locations. The total percentage of correctly assigned individuals was 92%.

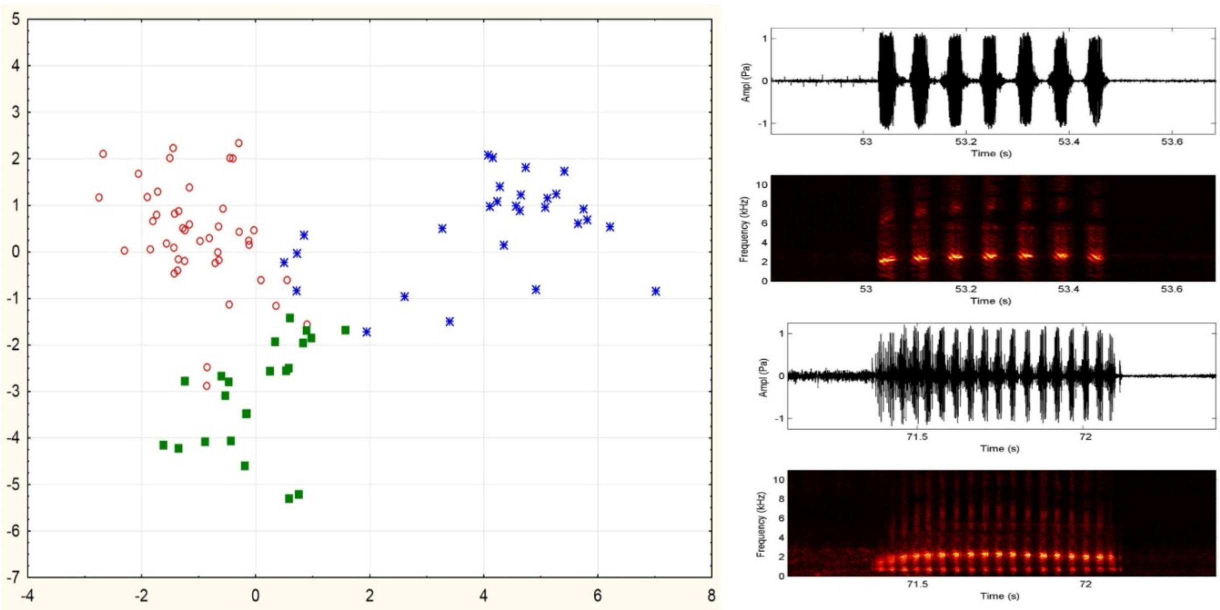



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**Fig. 2.** On the left – distribution pattern of calls from the three localities (square – Vurbitza 1, circle – Vurbitza 2, asterisk – Arkutino). On the right – a typical *P. ridibundus* call from Vurbitza 1 (above) and a typical *P. bedriagae* call from Arkutino (below).

Morphometric measurements were compared (where possible) to those of a comprehensive study on water frogs [23] and are presented on Table 3. Most of the measured traits were more consistent with those of *P. bedriagae* than *P. ridibundus*. On Figure 3 we present photographs of both the overall appearance as well as the callus internus of one of the specimens captured at Vurbitza.

**Table 2.** Measurements of the two specimens from Vurbitza (S1, S2), compared to data for *P. bedriagae* and *P. ridibundus* from [23]. For abbreviations, see Materials and methods.

| | S1 | S2 | *P. bedriagae* | *P. ridibundus* |
|---|---|---|---|---|
| **L.** | 62.12 | 59.02 | - | - |
| **F.** | 29.04 | - | - | - |
| **T.** | 29.26 | 29.84 | - | - |
| **D.p.** | 8.48 | 8.36 | - | - |
| **C.s.** | 16.18 | 17.42 | - | - |
| **C.int.** | 2.76 | 2.40 | - | - |
| **C.int.2** | 1.54 | - | - | - |
| **D.p./ C.int.** | 3.07 | 3.48 | 2.96 | 2.21 |
| **T./ C.int.** | 10.60 | 12.43 | 9.78 | 8.58 |
| **Ix** | 58.96 | 74.10 | - | - |
| **L./T.** | 2.12 | 1.98 | 2.16 | 1.92 |
| **L./ C.int.** | 22.50 | 24.6 | 21.16 | 16.57 |
| **L./ D.p.** | 7.32 | 7.06 | 7.15 | 7.49 |


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**Fig. 3.** A water frog specimen from Vurbitza river. Clearly visible are the dark green dorsal side with numerous black spots, the green tympanum and the small callus internus.

**Discussion**

Results from both the mating call analyses and the morphometric comparison confirm the presence of the Levant frog, *Pelophylax bedriagae*, in Bulgaria. Based on call characteristics we suggest that the species is more abundant in Arkutino, while in Vurbitza its presence probably fluctuates along the river. It has to be noted that calls from the two Vurbitza locations did not differ significantly in the key parameter of PG number, which was more similar to the one typical of *P. ridibundus* than to that of *P. bedriagae*. On the average, the mating call of the Marsh frog consists of 7 pulse groups per call [13], while that of the Levant frog consists of 12-20 [16], [8], [24]. We conclude that the Marsh frog is the predominant species in the two studied locations along the Vurbitza river, while the Levant frog is more common in Arkutino. Bioacoustic studies on the mating call of the Levant frog have proven that it remains remarkably uniform throughout the species range from the topotypic locality in Israel to Egypt and Syria, although some minor differences have been observed – i.e. in Syria and Egypt the parameter pulses per pulse group was significantly correlated with water temperature, while in Israel it was not [8]. For this reason, we did not use this parameter in our analyses but instead relied on the number of pulse groups per call, as well as call duration and the parameters related to frequency and energy, which are temperature-independent [8], [16] and so can be reliably used in comparison between different localities.

According to our analyses, the calls from Arkutino were slightly more similar to those from Vurbitza 1, rather than those from Vurbitza 2. This is somewhat contradictory, as on average Vurbitza 2 had calls with longer duration and more pulse groups than Vurbitza 1, although this difference was not statistically significant. This is probably due to the relatively small size of the samples from Arkutino and Vurbitza 2. Differences between frequency parameters could also be influenced by the size of the calling frogs [25]. What is certain is that calls from all three localities fall well within the described characteristics of the mating call of *P. bedriagae*.

In terms of their morphology, [23] describe the Levant frog (referred in their text as *Rana epeirotica* sp. n.) as having a uniform dorsal surface with many dark green spots with irregular shape, green


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tympanum without black border and a small callus internus with the shape of a truncated scalene triangle. This description is mostly consistent with the traits we observed on the two measured specimens, with the exception of the callus internus, which had somewhat intermediate form between those of *P. ridibundus* and *P. bedriagae*. Further research is needed in order to establish whether there are any hybrids between the two species on Bulgarian territory. In a comprehensive study [4] measured the following eight parameters: snout-vent length (SVL), femur length, tibia length, digitus primus length, callus internus length, maximal head width, snout-eye distance and tympanum diameter. While the authors do not provide the raw measurements (so we could not compare them to ours), they do conclude that these are very reliable characteristics for distinguishing between *P. bedriagae* and *P. ridibundus*.

From the current data on the distribution of the Levant frog, we can conclude that the species mainly occurs in regions with a mild climate and warmer temperatures. In contrast, the Marsh frog is adapted to colder conditions and therefore occupies more northern latitudes and in regions with predominantly continental climate (e.g. the type locality of Atyrau, localities in central Anatolia, etc.). However, there have been well-recorded exceptions -e.g. Lake Beyşehir in south-western Turkey, at 1 123 m.a.s.l., which is inhabited by *P. bedriagae* even though its climate would suggest the presence of *P. ridibundus* [9]. Various studies have indicated the presence of the Levant frog on the Maltese islands [24], the Denizli Province in Western Turkey [26] and, most importantly, even three localities in European Turkey [27]. Of these, the closest location to Bulgaria is Kiyiköy – a small coastal town at about 40km from the border. While Arkutino is further 30 km to the north, and the Vurbitza locations are even more distant to the north-west, it is very possible that frogs could have made this migration using the various waterways along the route. While indicative of the presence of the species in Bulgaria, the data presented here is still preliminary, and more detailed studies are needed in order to establish the exact distribution.

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