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## DISTRIBUTION AND POPULATION SIZE OF THE MOOR FROG (*RANA ARVALIS*) IN THE RÂUL NEGRU/FEKETEÜGY RIVER BASIN

**Key words:** amphibians, egg clumps, habitat, range limit, threatened population

### Introduction

The moor frog (*Rana arvalis* Nilsson, 1842) is a species widely distributed in Eurasia, from the Netherlands to Baikal Lake and Yakutia. Romanian populations represent the southernmost occurrence of the species.<sup>1</sup> Here, the moor frog is considered one of the rarest amphibians and is distributed in four separate geographical regions: the western lowlands along the Hungarian border, northern Moldavia, the Transylvanian Plateau and depressions from the Eastern Carpathians.<sup>2</sup> In this study, we present data from this latter region, namely the Râul Negru/Feketeügy River Basin.

Presence of the moor frog in the study area was first reported by Dely<sup>3</sup> from Reci/Réty, soon after which the biology and ecology of this population was also briefly studied by Fuhn<sup>4</sup>. Because of the well-known works of Fuhn<sup>5</sup> and the fame of the Mestecănișul de la Reci/Rétyi-Nyír Nature Reserve, this site has been a well-known occurrence of the species, and was also included in a European-scale genetic study.<sup>6</sup> Despite of this, the population has never undergone detailed ecological studies. Fuhn<sup>7</sup> recorded the species from several localities of our study area, namely Reci/Réty, Covasna/Kovászna, Târgu Secuiesc/Kézdivásárhely, Cătălina/Szentkatolna, Imeni/Imecsfalva and Páva/Páva (the latter now part of Zăbala/Zabola). Some of these populations could have disappeared since, as suggested by Sas et alii<sup>8</sup>, however such statements must be treated with caution, because of the lack of surveys undertaken in subsequent decades. In more recent times, in an attempt of mapping the amphibians of Covasna County, Csata & Csata<sup>9</sup> recorded the moor frog in 6 UTM-squares of 2×2 km of the depression, in the surroundings of Reci/Réty, Sântionlunca/Szentivánlaborfalva, Țufalău/Cófalva

and Târgu Secuiesc/Kézdivásárhely localities. The species was also identified in the Olt River Basin near Prejmer/Prázsmár as part of a faunistic survey (Sos, unpublished).

Data on the herpetofauna of the region are also found in Ghira et alii<sup>10</sup>, including, however, numerous dubious records, owing most probably to the dominating data collection method of questionnaire survey, rather than field studies. Based on the above, the credibility of data from this work is strongly questioned.<sup>11</sup>

The aims of the present paper are: 1. to evaluate the current distribution of the moor frog in the Râul Negru/Feketeügy River Basin, 2. to delimit populations and 3. to assess population size.

### Material and methods

#### *Description of the study site*

The Râul Negru/Feketeügy River Basin lies in the south-eastern part of Transylvania. This river is the main tributary of the Olt River in its Transylvanian sector, with its source in the Nemira/Nemere Mountains. It has a total length of 46 km, and forms a depression of some 700 km<sup>2</sup>. The area is flat, with altitudes ranging between approximately 500 and 620 m. The left bank of the river is characterized by a relatively large portion of marshland, oxbows and wet meadows, in this respect sharing similarities with the two mountain depressions of the Romanian Eastern Carpathians, the Giurgeului/Gyergyó<sup>12</sup> and the Ciuc/Csik Depressions<sup>13</sup>, however having a milder climate. It features relatively large areas of grassland, alternating with small-parcel arable land, however plot surfaces show an increase as a consequence of agriculture intensification. The dominating soil types are fluvi-eutric gleysol, haplic phaeozem and luvic

<sup>1</sup> GASC, Jean-Pierre et alii 1997.

<sup>2</sup> SAS István et alii 2008.

<sup>3</sup> DELY Olivér György 1953.

<sup>4</sup> FUHN, Ion E. 1956.

<sup>5</sup> FUHN, Ion E. 1956, 1960.

<sup>6</sup> RAFIŃSKI, Jan – BABIK, Wiesław 2000.

<sup>7</sup> FUHN, Ion E. 1960.

<sup>8</sup> SAS István et alii 2008.

<sup>9</sup> CSATA Edit – CSATA Zoltán 1997a, 1997b.

<sup>10</sup> GHIRA, Ioan et alii 2002.

<sup>11</sup> See also DEMETER László – HARTEL Tibor 2007, HEGYELI Zsolt 2011.

<sup>12</sup> DEMETER László et alii 2012, HEGYELI Zsolt 2011.

<sup>13</sup> DEMETER László et alii 2006.

phaeozem (Full Soil Code 1974 FAO – European Soil Portal).

#### *Distribution survey*

Field investigations were carried out mostly in 2011 and 2012, and to a lesser extent in 2008 and in 2013. Breeding sites were identified by surveying potentially suitable breeding habitats (temporary and permanent ponds, ditches, marshes and oxbows) for breeding specimens (by visual observation or after call of breeding males) or egg clumps, during the breeding period (usually between 20 March and 10 April).

Distribution outside the breeding period was established by means of night and daylight (latter mostly during rainy weather) transect surveys in potentially suitable terrestrial habitats (marshlands, wet meadows and riparian habitats).

#### *Separation of populations*

Delimitation of populations was performed based on a) continuity of potential moor frog habitat (such as marshlands or wet grasslands) as well as b) distance of known breeding ponds from each another within the continuous habitat. Similarly to Demeter & Mara<sup>14</sup>, who included breeding ponds closer than 500 m to each other as belonging to one population, we used a maximum distance of 750 m. In four cases, however, ponds with distances of up to 1000 m were included in the same population, provided that there were known terrestrial occurrences or continuous potential habitat connecting them.

#### *Population size estimation*

Population size was estimated by the method of egg clump counts in the breeding period<sup>15</sup>. Egg clumps of the three brown frog species (i.e. moor frog, agile frog (*Rana dalmatina*) and common frog (*Rana temporaria*) were distinguished based on size, shape as well as characteristics of their deposition, as described by Demeter et alii<sup>16</sup>. In lack of egg clumps (usually in the beginning of the breeding period) the number of vocalizing males was assessed visually, using binoculars<sup>17</sup> or (rarely, in lack of visibility) acoustically<sup>18</sup>. Since each female moor frog lays only one egg clump in one spawning season, the number of egg clumps should equal the number of reproducing females. In lack of data about the sex ratio of adults, similarly to other studies<sup>19</sup> a sex

ratio of 1:1 was assumed, hence population size was expressed by doubling the number of egg clumps or vocalizing males (always the higher number of the two), which should equal the minimum number of reproducing individuals within the population (also called adult population size<sup>20</sup>). In terrestrial habitats no population size estimation was performed.

It is important to mention, however, that due to the field surveys covering different stages of the reproduction, in some spawning sites a certain ratio of males or egg clumps could not be detected (e.g. in the beginning of the spawning period, when not all egg clumps are yet laid, or not all males have entered the water); an underestimation of the population size is hence expected.

Spatial data were recorded using a GPS receiver (Garmin GPSMap 60CSx) and processed using the ArcMap software (version 10.1, ESRI).

## Results

During our study egg clumps or breeding moor frog individuals were identified in 37 separate spawning water bodies along the Râul Negru/Feketeügy River. A total number of 2466 moor frog egg clumps were identified (*Table 1*). The mean number of egg clumps per pond was  $70.45 \pm 19.60$  (SD = 115.96, range 1-40, n = 35). In terrestrial habitats moor frog individuals were encountered in 11 separate sites.

Based on the continuity of potential habitat and the distance between individual spawning ponds, 10 individual populations were identified. Two other sites provided observations of terrestrial moor frog individuals only, however these were also identified as separate populations (*Table 2, Fig. 1*). Size of potential habitats was measured on satellite images and based on our GPS data, and averaged 128.9 ha. The mean number of ponds where breeding moor frogs were found in distinct populations was 3.7 (range 1-9) (*Table 1*).

Assessment of population size was performed in the case of 10 populations, however, only 7 estimates are considered relevant, as in the remaining 3 cases (Ozun/Uzon, Lunga/Nyújtód, Hătuica/Hatolyka) not all available habitats were checked, hence the estimate should be considered preliminary. In further two sites (Brateş/Barátos, Zăbala/Zabola) only a few individuals have been observed outside the breeding

<sup>14</sup> DEMETER László – MARA Gyöngyvér 2005.

<sup>15</sup> GLANDT, Dieter 2008, LOMAN, Jon – ANDERSSON, Gunilla 2007, LYAPKOV, Sergey M. 2008.

<sup>16</sup> DEMETER László et alii 2012.

<sup>17</sup> STANKOVIĆ, David – CIPOT, Maja 2014.

<sup>18</sup> DEMETER László et alii 2012.

<sup>19</sup> E.g. DEMETER László – MARA Gyöngyvér 2005, DEMETER László – BENKŐ Zoltán 2008, STANKOVIĆ, David – CIPOT, Maja 2014.

<sup>20</sup> DEMETER László et alii 2012.

period, not allowing for population size estimates. The mean population size was 722 adults (range 258–2430,  $n=7$ ), with the Reci/Réty population proven to be the largest, followed by the Cătălina/Szentkatolna and the Telechia/Orbaitelek populations (Table 1, Fig. 1). The total number of adult moor frogs in the study area (considering 10 populations) was assessed at 5176 individuals (Table 1). Projecting our records in UTM grids, 22 grids of 2×2 km provided moor frog records, while other suitable habitats with no moor frog records so far cover an additional 12 grids (Table 2).

## Discussion

Among the 12 identified populations, only 3 are located on the right bank of the Râul Negru/Feketeügy, which is not surprising, considering the general scarcity of available habitats here, compared to the left bank of the river. Although we have separated 12 populations based on habitat availability and distribution in the breeding period, the possibility of metapopulation systems, as described by Marsh & Trenham<sup>21</sup> should not be ruled out.

Estimated population sizes were similar to, but generally higher than those from the north-western part of Romania<sup>22</sup>. At the same time, they showed similarity to population sizes from the Ciuc/Csík Depression<sup>23</sup>, and the largest (Reci/Réty) population also showed a similar estimate to the largest population from the Ciuc/Csík Depression described by Demeter & Benkő<sup>24</sup> (1195 and 1400 egg clumps, respectively). Nonetheless, our estimates were considerably lower compared to the largest population known in Romania (from the Giurgeului/Gyergyó Depression), where Demeter et alii<sup>25</sup> counted 3244 egg clumps in one site.

The quality of population estimates may show some variation due to the differences in survey effort, spawning habitat availability and weather conditions. E.g. in the Reci/Réty population the survey was performed in late March 2008 without covering all potential breeding sites; thus, population size could be considerably higher. In case of the Țufalău/Cófalva and Tinoasa / Sârfalva populations, field work was carried out both in 2011 and 2012, however, without visiting the same spawning sites in the two years. Despite this difference, data from the two survey years were not combined, instead the higher figure was provided as population size estimate,

given that individuals might use various spawning sites in different years. Due to the extreme drought and consequent scarcity of available breeding ponds in the spring of 2013, only a few potential spawning sites were located, thus the Ozun/Uzon population (which in less dry years may benefit from abundant spawning sites, given the general habitat availability) is likely underestimated.

Generally, the use of terrestrial habitats and movements to spawning sites by the moor frog are insufficiently understood<sup>26</sup>. Consequently, further studies focusing on terrestrial habitats in our study area could provide more accurate data, particularly about dispersal of individuals and population limits.

Based on the Hungarian ÁNÉR system<sup>27</sup>, most spawning habitats can be included in categories B4 (tussock sedge communities), BA (fine scale mosaic or zonation of marsh communities) and D34 (mesotrophic wet meadows). The latter habitat type is usually utilized as pasture in the study area, and is also thought to be an important terrestrial habitat of the species. Populations from Reci/Réty live mostly in J1b (birch mire forests), though they are also found in habitats outside the forest.

Some individuals were located in quite unusual habitats. In the case of the Hătuica/Hatolyka population, individuals were found in one atypical breeding site (an oxbow) together with common frogs; a small chorus (of approximately 10 male moor frogs and slightly more common frog individuals) was heard in this habitat with a water depth of more than 100 cm (not allowing for egg clump count) and lacking shallow water macrophytes. Based on our experience, previously only common frogs have been encountered in such habitats during the breeding period. In Zăbala/Zabola a male individual still having a partial nuptial colour was found in an angling lake soon after the breeding period. At the time, this lake held pikes (*Esox lucius*), a likely cause for which not even the abundant common frog population used it for spawning. During the terrestrial period, another adult individual was found at a distance of 1.1 km from the nearest potential habitat, in a garden from Zăbala/Zabola village.

In spawning sites from our study area, moor frogs were found associated with all but one – i.e. common tree frog (*Hyla arborea*) – co-occurring amphibian species: northern crested newt (*Triturus cristatus*), Transylvanian smooth newt (*Lissotriton vulgaris*

<sup>21</sup> MARSH, David M. – TRENHAM, Peter C. 2001.

<sup>22</sup> SAS István et alii 2006.

<sup>23</sup> DEMETER László – MARA Gyöngyvér 2005.

<sup>24</sup> DEMETER László – BENKŐ Zoltán 2008.

<sup>25</sup> DEMETER László et alii 2012.

<sup>26</sup> MARSH, David M. – TRENHAM, Peter C. 2001.

<sup>27</sup> BÖLÖNI János et alii 2011.

*ampelensis*), yellow-bellied toad (*Bombina variegata*), common spadefoot (*Pelobates fuscus*), common toad (*Bufo bufo*), green toad (*Bufo viridis*), agile frog, common frog, edible frog (*Pelophylax esculentus*), pool frog (*Pelophylax lessonae*) and marsh frog (*Pelophylax ridibundus*), whereas in terrestrial habitats they were encountered together with common spadefoot, green toad and common frog. Among these, smooth newt and common frog were found in 5, common spadefoot in 4, while northern crested newt in 3 populations in the same ponds with breeding moor frogs.

Mixed breeding choruses of moor frogs and common frogs were observed in 4 breeding ponds, in 4 different populations. Some of these choruses were dominated by moor frog males, while others were common frog-biased. To our knowledge, our study area is the only region known in Romania where all three brown frog species co-occur in the same habitats, sometimes even using the same spawning water bodies.

In the conditions of the Râul Negru/Feketeügy River Basin, the breeding period of moor frogs lasted from the last third of March to mid-April, depending on weather conditions, and based on our data was preceded by that of agile frogs and common frogs, however sometimes being synchronous with the latter.

Over the study years, various threats have been detected in the study area. Among these, natural drought it was a frequent one, although this is often due to the destruction of the water balance, hence it can be considered man-made. Dry springs of recent years might have had a serious impact on breeding amphibian populations in the area. Drainage and canalization of marshlands and swamps have been identified in several sites, similarly to the use of ditches

for irrigation purposes, both of which contribute to the loss of breeding amphibian habitat. Springtime burning of boggy wetlands and reed habitats takes place yearly, while the ploughing of meadows and wet grasslands or the creation of willow plantations for bioenergy on grasslands are aggressive, yet common forms of land expropriation detected in the area. Human consumption has been only proven to affect the common frog in the study area, but it is reasonable to assume that in mixed breeding habitats also moor frogs fall victim to this illegal act. Road mortality was identified as a threat to some amphibian populations in the area. The presence of invasive alien plant species in several wetlands<sup>28</sup> can be regarded as a general threat to these habitats.

The moor frog population of the Râul Negru/Feketeügy River Basin lives at the southern limit of the species range, and can be found in ever-shrinking habitat types, in several isolates. Rafiński & Babik<sup>29</sup> identified the Reci/Réty population as the one with the lowest genetic variation compared to 13 other European populations, a likely result of its isolation.

Even though four of the identified populations lie entirely or partially within protected areas (*Mestecănișul de la Reci și Bălțile de la Ozun-Sântionlunca/Rétyi Nyír és Uzon-Szentivánlaborfalvi tavak természetvédelmi terület* nature reserve as well as Natura 2000 sites *ROSCI0111 Mestecănișul de la Reci* and *ROSCI0374 Râul Negru*), this alone does not guarantee the effective conservation of the moor frog. There is an urgent need to designate protected areas exclusively or primarily for the conservation of moor frog habitats, and to enforce practical protection within the above protected areas. The use of the moor frog as a flagship species for amphibian and wetland protection would also be beneficial in the region.

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<sup>28</sup> E.g. KOVÁCS J. Attila 2006.

<sup>29</sup> RAFIŃSKI, Jan – BABIK, Wiesław 2000.

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## **A mocsári béka (*Rana arvalis*) elterjedése és állománymérete a Feketeügy mentén**

(Kivonat)

A mocsári béka (*Rana arvalis*) elterjedését 2008 és 2013 között vizsgáltuk a Feketeügy folyó völgyében, elsősorban a szaporodási időszakban. A fajt 37 szaporodó víztestből mutattunk ki, és az alkalmas élőhelyek elérhetősége, valamint a szárazföldi megfigyelések alapján 12 különálló populációját azonosítottunk. Az állományok méretét a petecsomók és az éneklő hímek száma alapján becsültük, eszerint a vizsgált terület populációját 5176 felnőtt egyedre tettük. A legnagyobb egyedszámot a rétyi állomány esetében találtuk. A mocsári béka élőhelyei folyamatosan hanyatlanak, ami sürgős védelmi intézkedéseket követel a faj itteni fennmaradásának biztosítása érdekében.

## **Distribuția și mărimea populațională a broaștei de mlaștină (*Rana arvalis*) în valea Râului Negru**

(Rezumat)

Distribuția broaștei de mlaștină (*Rana arvalis*) a fost cercetată în perioada 2008–2013 de-a lungul Râului Negru. În perioada de reproducere, specia a fost detectată în 37 ochiuri de apă, iar combinat cu semnalările din faza terestră și distribuția habitatelor, acestea au fost asociate cu 12 populații distincte. Mărimea populațiilor a fost evaluată pe baza numărului pontelor și a masculilor observați în timpul reproducerii, și a fost estimată la 5176 adulți în zona de studiu. Populația de la Reci s-a dovedit a fi cea cu numărul de indivizi cel mai ridicat. Habitatele speciei sunt într-un declin continuu, pentru care sunt necesare acțiuni urgente de conservare în vederea asigurării supraviețuirii broaștei de mlaștină în zona de studiu.

Distribution and population size of the moor frog (*Rana arvalis*) in the Râul Negru/Feketeügy River Basin

Table 1 Egg clump count and population size data in the breeding period of moor frogs (*Rana arvalis*) along the Râul Negru

1. táblázat A petecsomó-számlálás és az állománybecslés adatai a mocsári béka (*Rana arvalis*) szaporodási időszakában a Feketeügy mentén

Population <i>Populáció</i>	Number of spawning waters <i>Szaporodó vizek száma</i>	Number of egg clumps <i>Petecsomók száma</i>	Mean number of egg clumps/spawning water <i>Átlag petecsomó-szám/ szaporodó víztest</i>	Minimum assessed number of individuals <i>Legkisebb becsült egyedszám</i>
Reci/Réty	9	1195	132.8	2430
Tinoasa/Sárfalva	4	354	88.5	708
Telechia/Orbaitelek	3	321	107.0	642
Mártineni/Kézdimartonfalva	5	205	41.0	410
Peteni/Székelypetőfalva	4	164	41.0	328
Țufalău/Cőfalva	3	68	22.7	280
Tamaşfalău/Székelytamásfalva	4	109	27.3	258
Ozun/Uzon	2	33	16.5	66
Lunga/Nyújtód	2	17	8.5	34
Hătuica/Hatolyka	1	-	-	20
Mean/Átlag	3.7	274	-	517.6
Total/Össz.	37	2466	-	5176

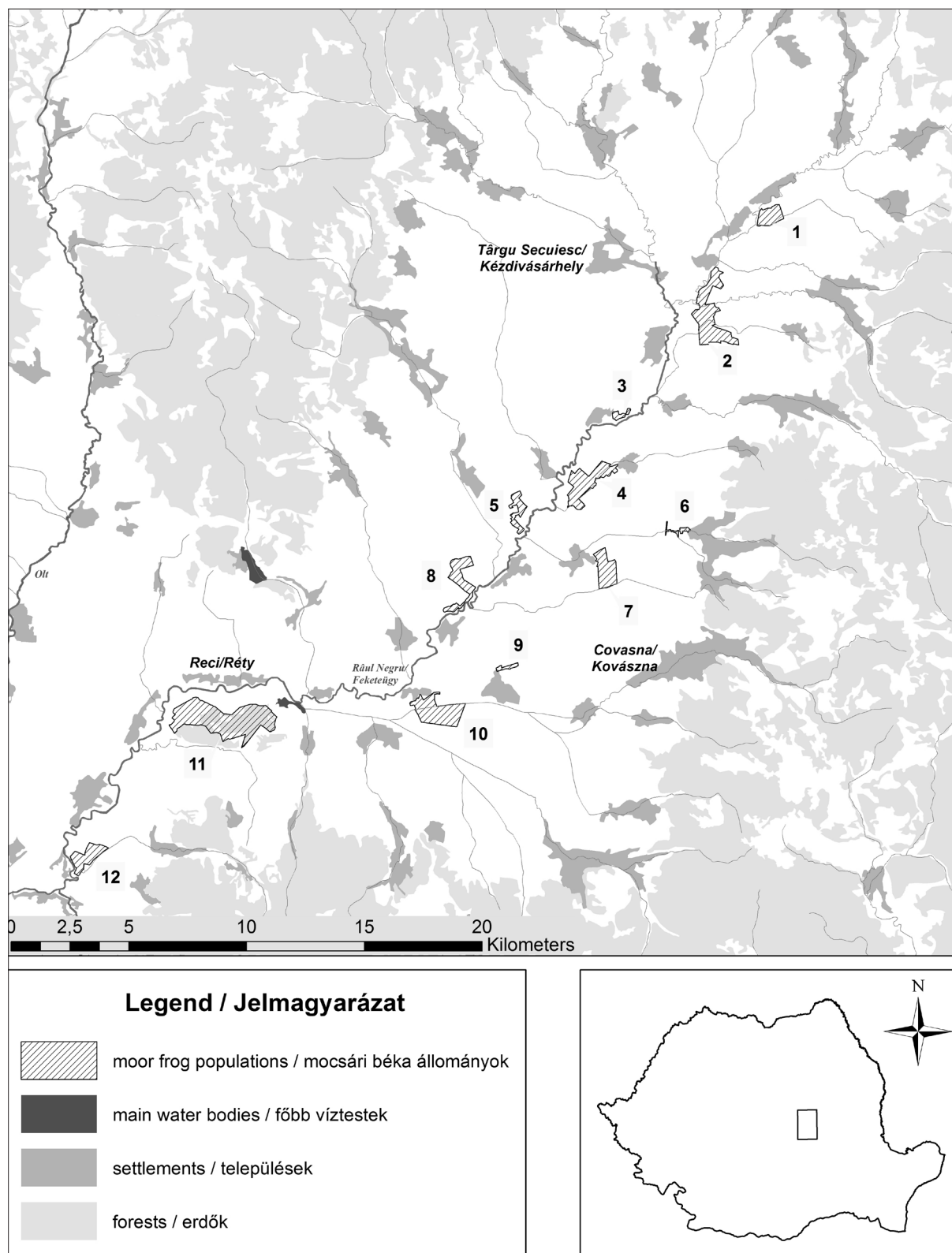
Table 2 Distribution data of moor frog (*Rana arvalis*) populations along the Răul Negru

\*Records of individual moor frogs, \*\*Occurrence of potential habitats

2. táblázat A Feketeügy mentén azonosított mocsári béka (*Rana arvalis*) állományok elterjedési adatai

\*Mocsári béka észlelések, \*\*Alkalmos élőhelyek elterjedése

Population <i>Populáció</i>	Area of suitable habitats (ha) <i>Alkalmos élőhelyek területe (ha)</i>	Administrative unit <i>Közigazgatási terület</i>	Occurrence in 2 × 2 km UTM grids <i>Előfordulás 2 × 2 km-es UTM-rácsokban</i>
Reci/Réty	469	Reci/Réty, Ozun/Uzon	ML1.1 7.2*, ML1.2 7.2, ML1.3 7.2, ML1.2 7.3**, ML1.4 7.2
Tinoasa/Sárfalva	241	Târgu Secuiesc/Kézdivásárhely, Ghelinița/Gelence	ML3.3 9.1, ML3.4 9.0, ML3.3 9.0
Telechia/Orbaitelek	112	Dalnic/Dálnok, Boroșneu Mare/Nagyborosnyó, Brateș/Barátos	ML2.3 8.0, ML2.2 7.4, ML2.3 7.4, ML2.2 8.0
Mărtineni/Kézdimartonfalva	56.2	Cătălina/Szentkatolna	ML2.4 8.2, ML2.4 8.1
Peteni/Székelypetőfalva	168	Zăbala/Zabola	ML3.0 8.2, ML3.1 8.2, ML3.0 8.1
Țufalău/Cófalva	189	Boroșneu Mare/Nagyborosnyó, Brateș/Barátos	ML2.2 7.3, ML2.2 7.2, ML2.3 7.2
Tamașfalău/Székelytamásfalva	107	Zăbala/Zabola	ML3.1 8.0, ML3.1 8.1
Ozun/Uzon	91	Ozun/Uzon, Chichiș/Kökös	ML0.4 6.4, ML1.0 7.0, ML1.0 6.4
Lunga/Nyújtód	69.9	Târgu Secuiesc/Kézdivásárhely	ML3.4 9.3, ML4.0 9.3, ML3.4 9.2, ML4.0 9.2
Hătuica/Hatolyka	16.7	Cătălina/Szentkatolna	ML3.1 8.3
Brateș/Barátos	13.2	Brateș/Barátos	ML2.3 7.3, ML2.4 7.3
Zăbala/Zabola	13.7	Zăbala/Zabola	ML3.2 8.1, ML3.3 8.1



**Figure 1.** Moor frog (*Rana arvalis*) populations along the Râul Negru, from north to south: 1 – Lunga, 2 – Tinoasa, 3 – Hătuica, 4 – Peteni, 5 – Mărtineni, 6 – Zăbala, 7 – Tamaşfalău, 8 – Telechia, 9 – Brateş, 10 – Ţufalău, 11 – Reci, 12 – Ozun  
**1. ábra** Mocsári béka (*Rana arvalis*) állományok a Feketeügy mentén, északról délre: 1 – Nyújtód, 2 – Sársfalva, 3 – Hatolyka, 4 – Székelypetőfalva, 5 – Kézdimartonfalva, 6 – Zabola, 7 – Székelytamaşfalva, 8 – Orbaitelek, 9 – Barátos, 10 – Cófalva, 11 – Réty, 12 – Uzon



Figure 2. Moor frog (*Rana arvalis*) individuals. a. Terrestrial individual from Zábala, b. Male and female from Mártoneni in the breeding period, c. Female from Mártoneni, d. Terrestrial individual from Cătălina

2. ábra Mocsári béka (*Rana arvalis*) egyedek. a. Szárazföldi példány Zaboláról, b. Hím és nőstény példány a szaporodási időszakból, Martonfalváról, c. Nőstény példány Martonfalváról, d. Szárazföldi példány Szentkatolnáról



Figure 3. Typical moor frog (*Rana arvalis*) breeding habitats. a. Ţufălău, b. Tamaşfalău, c. Reci, d. Moor frog egg clumps in a pond from Peteni

3. ábra Jellemző mocsári béka (*Rana arvalis*) szaporodó élőhelyek. a. Cőfalva, b. Székelytamásfalva, c. Réty, d. Mocsári béka petecsomók egy székelypetőfalvi tócsában