

## A reassessment of the northeastern distribution of *Rana dalmatina* (Bonaparte, 1840)

Maciej Bonk<sup>1,2</sup>, Stanisław Bury<sup>3</sup>, Sebastian Hofman<sup>1</sup>, Jacek M. Szymura<sup>1</sup> and Maciej Pabijan<sup>1,\*</sup>

**Abstract.** This contribution depicts the northeastern distribution of *Rana dalmatina* based on new data collected in 2005-2011 and a review of known localities. The northeastern peripheral range of this species contains six centers of occurrence in Poland that are probably isolated from each other. *Rana dalmatina* is most abundant (in terms of number of breeding sites) in a ca. 3000 km<sup>2</sup> area of the southern Sandomierz Basin and adjacent Carpathian Foothills in southeastern Poland. However, even here (at the regional scale) the range seems fragmented: we document three forested areas with 44, 17 and six breeding sites. In addition, we report another cluster of previously unknown breeding sites close to the San River at the northern margin of the Western Carpathian Mts., providing evidence for a more extensive eastern range of this species. Based on a lack of variation in a 710 bp fragment of mtDNA, we suggest that these *R. dalmatina* populations were established recently and probably from a single, southern source population. In the context of the known distribution in adjacent countries, we outline three possible colonization scenarios for this species: from a formerly more extensive distribution in the west, a possible eastern expansion along the Dniester River in Ukraine, or from the south through the Moravian Gate, a natural depression between the Sudetes and Carpathian Mts.

**Keywords.** Anura, Ranidae, peripheral populations, mtDNA, fragmentation, breeding habitat.

### Introduction

The borders of species ranges have received considerable attention in recent years due to a reappraisal of their evolutionary potential and conservation value (e.g. Gibson, van der Marel and Starzomski, 2009). Contemporary emphasis on the significance of peripheral populations stems from two interrelated observations: (i) peripheral populations may contain more dispersive individuals (Parmesan, 2006), (ii) they possibly contain genotypes that are better suited to colonize new habitats because they already exist in conditions similar to those just outside the current range (e.g. Ficetola and De Bernardi, 2005; Hamilton and Eckert, 2007). The northern range margins of Palearctic species may be particularly important due to their response to projected poleward biome forcing as a result of global climate change (Williams, Jackson and Kutzbach, 2007; Gibson, van der Marel and Starzomski, 2009). Northward expansions are expected for a number of European amphibian and reptile species (Araújo,

Thuiller and Pearson, 2006). Thus it is important and urgent to document the fine-scale distribution, habitat, life history and demography of peripheral populations.

The agile frog, *Rana dalmatina* Bonaparte, 1840 is a widely distributed terrestrial ranid species in Europe. Its continuous range extends from the Atlantic Coast (with the exception of most of the Iberian Peninsula) to the Balkan Peninsula where it reaches its southern limits. The northernmost populations, located in Sweden, Denmark and central Germany, are outside of its continuous range (Gasc et al., 1997). The agile frog reaches its northeastern distribution limit in a few scattered populations in southeastern Poland (Błachuta and Jabłoński, 1986; Rafiński, Szymura and Smykla, 1987; Szymura, 1994, 2003; Szymura and Rafiński, 1997). These localities are situated north of the Western Carpathian Mountains, a prominent landscape feature in this part of Central Europe. The number of populations, abundance at individual sites and the precise distribution of the agile frog in this region are based on only a few localities (most recently summarized by Szymura, 2003); much of the original literature is not readily accessible to the wider herpetological community.

Until the late 1980s the occurrence of *R. dalmatina* in Poland was controversial (Juszczyk, 1987). Only one observation was believed to be well documented and it was based on a museum sample from 1918 presumably collected near Wrocław (Borkin, 1977; Juszczyk, 1987). However, this locality has not been

1 Department of Comparative Anatomy, Institute of Zoology, Jagiellonian University ul. Gronostajowa 9, 30-387, Kraków, Poland

2 Department of Entomology, Institute of Zoology, Jagiellonian University ul. Gronostajowa 9, 30-387, Kraków, Poland

3 Institute of Environmental Sciences, Jagiellonian University ul. Gronostajowa 7, 30-387, Kraków, Poland

\*Corresponding author; email: maciej.pabijan@uj.edu.pl

recently verified. Much later, Błachuta and Jabłoński (1986), relying on morphology, reported this species from southeastern Poland (near the city of Przemyśl). Rafiński, Szymura and Smykla (1987) and Rafiński and Szymura (1991) documented several new localities using allozyme electrophoresis. Subsequently, this species was documented at three other sites in the foothills of the Carpathian Mts. or in their immediate vicinities (Szymura and Rafiński, 1997; Szymura, 1994, 2003; Bartoń and Rafiński, 2006; Starzyk and Durak, 2007). Recently, a new breeding site of *R. dalmatina* was found in south-central Poland, on the border of the Czech Republic (Najbar, Vlček and Šuhaj, 2011). As all amphibian species in Poland, *R. dalmatina* has been given legal protection figuring in the Polish Red Data Book of Animals as near threatened (NT; Rafiński and Szymura, 2001). Moreover, it is protected by the Habitats Directive (appendix IV; requiring protection) and Bern Convention (appendix II; species under strict protection). It is also listed in the IUCN Red List as least concern (LC) with decreasing numbers of populations.

The aim of this contribution is to present recent data on the occurrence of the agile frog in Poland. We have extensively surveyed potential *R. dalmatina* habitat in an area of ca. 3000 km<sup>2</sup> and significantly expanded the known distribution of this species in southeastern Poland. In addition, we document another cluster of previously unknown breeding sites close to the Bieszczady Mountains in southeastern-most Poland, providing evidence for a more extensive range of *R. dalmatina* at its northeastern periphery. Moreover, we contribute observations on terrestrial and aquatic habitat and preliminary data on abundance at some of the breeding sites. We conclude by placing these populations in the context of the known distribution in adjacent countries and suggest several scenarios for the origin of this species in the northeastern part of its range.

## Materials and Methods

### Study area

New locality records for *R. dalmatina* in Poland were compiled from an ongoing amphibian survey and monitoring effort in south-central Poland (Pabijan, 2009; Bonk and Pabijan, 2010) and unpublished data collected between 2005 and 2011. Most of the surveyed area was restricted to the Sandomierz Basin and adjacent parts of the Carpathian Foothills (Figs. 1-2), an area of approximately 90 km x 35 km. The Sandomierz Basin (Kotlina Sandomierska) is a large lowland area delimited by the Małopolska Upland at the northern edge and Carpathian Foothills in the south. The altitude does not exceed 300 m asl; the altitudinal range of the

surveyed breeding sites ranged between 170-230 m asl. Potential breeding sites were mostly preselected using Google Earth and aerial maps ([www.geoportal.gov.pl](http://www.geoportal.gov.pl)). The timing of surveys coincided with the breeding season of *R. dalmatina* in Poland (March through April). However, some sites were visited also between May and July. We surveyed mostly forested areas, as these are in line with the habitat preferences of the species and our previous field experience. All lowland habitats are remnants of former continuous woodland fragmented in historical times and encompass mostly mixed forests (*Abies alba*, *Alnus spp.*, *Betula pendula*, *Fagus sylvatica*, *Pinus silvestris*, and *Quercus spp.*). Although fragmented, these lowland woodlands are relatively large in size. We also report five breeding sites from southeastern Poland, in a small mountain range known as the Sanok-Turka Range (Góry Sanocko-Turczańskie) located at the northern margin of the Western Carpathian Mountains. These localities are situated between the villages of Myczkowce and Uherce Mineralne. This area is dominated by hills and low mountains (between 350-550 m asl) interspersed by fast flowing tributaries of the San River. Forest cover is extensive, dominated by *Fagus sylvatica*, *Alnus spp.* and *Salix spp.* in stream valleys.

### Species detection in the field

The presence of adults and egg batches was noted. Adult *R. dalmatina* were generally visually identified by examining the length of the leg relative to snout-vent length and other morphological characteristics such as tympanum size, coloration, and coloration of the eye (in the three brown frogs present in southern Poland, the lower half of the iris is usually darker in *R. dalmatina*). All adult or juvenile frogs were released in the place of capture. Egg batches were identified by the characteristic manner of egg deposition in this species. In contrast to *R. temporaria* and *R. arvalis* which lay their eggs in aggregations in the shallowest parts of ponds, clutches of *R. dalmatina* eggs are usually dispersed throughout the water body (Guyétant, 1997; Bartoń and Rafiński, 2006). Moreover, freshly deposited eggs are mostly attached to vertical vegetation a few centimeters below the surface of the water and have a globular shape (Fig. 3). Older clutches in most cases become green due to algae development in their gelatinous envelopes (Baumgartner *et al.*, 1996). A typical, algae-colonized clutch of *R. dalmatina* eggs is shown in Fig. 3.

### Molecular identification

We confirmed the identity of single eggs from putative *R. dalmatina* clutches taken from 11 of the new localities by amplifying and sequencing a fragment of the mitochondrial genome using the primers ND1daL (5' GACCTNACNGAAGGTGAATCTG 3') and ND1daH (5' AAAATCAGCGGGTTRAATATCAC 3'). These primers amplify an approximately 1990 bp fragment of the mitochondrial genome of *R. dalmatina*, *R. arvalis* and *R. temporaria* encompassing the ND1, tRNA-Ile, tRNA-Gln, tRNA-Met, ND2, tRNA-Trp, tRNA-Ala, tRNA-Asn, L-strand origin of replication, tRNA-Cys, and tRNA-Tyr genes. This mtDNA fragment is being used in a wider phylogeographical investigation (S. Hofman, J.M. Szymura, in prep.). Amplifications were performed in 20 µL reac-

tion mixtures containing approximately 20 ng of genomic DNA, 2  $\mu$ L of the 10  $\times$  polymerase chain reaction (PCR) buffer (Fermentas), 2.5 mM MgCl<sub>2</sub>, 0.2 mM of each dNTP, 1  $\mu$ M of both the forward and reverse primers and 0.25 U of *Taq* polymerase (Fermentas) with the cycling scheme: 94 °C for 2 min, 55 °C for 45 s, 72 °C for 4 min, followed by 34 cycles at 94 °C for 40 s, 55 °C for 45 s, 72 °C for 200 s, and the final extension at 72 °C for 5 min. We sequenced 710 bp using the ND1daL primer in 16 *R. dalmatina* individuals from 11 localities (Fig. 4). As in earlier allozyme surveys, we also included sequences from sympatric *R. arvalis* (Drwinia 3723: 50.1011°N; 20.4063°E) and *R. temporaria* (Lubzina 3719: 50.0559°N, 21.52321°E). Amplicons, purified with Clean-Up columns (A & A Biotechnology), were subject to cycle sequencing using the Big-Dye Terminator Kit (ABI). After removing terminators with ExTerminator columns (A & A Biotechnology) sequencing reaction products were separated on an ABI Prism 3100 Avant Genetic Analyser. We used Mega 5.03 (Tamura et al., 2011) for basic sequence analyses and to confirm the species identity of all eggs assigned to *R. dalmatina* (on the basis of clutch morphology) by constructing a bootstrapped neighbor-joining tree that included the outgroups.

#### Literature survey

We provide an account of published occurrence data for *R. dalmatina* from countries adjacent to southern Poland in order to place the distribution of Polish localities into the broader context of the continuous distribution of this species. We gathered data from the eastern part of the Czech Republic (Moravec, 1994; Zavadil, 1997), Slovakia (Lác, 1968; Zavadil, 1997), northwestern Ukraine (Tarašćuk, 1959; Reminnyi, 2007; Pisanets and Reminnyi, 2008). A graphical summary, illustrating the northeastern distribution of *R. dalmatina*, is shown in Fig. 1. Because the original data was inferred from maps drawn at different scales, published for various purposes, and using data collected throughout the 20th century, Fig. 1 should be considered as only an approximation of the known range of this species at its northeastern fringe, and be used as a guide in future surveys aimed at filling the gaps in the knowledge of the distribution of this species.

## Results

#### New breeding sites in southeastern Poland

A total of 54 new breeding sites of *R. dalmatina* were found in 2005–2011 in southeastern Poland (Table 1); six additional localities provided by Bartoń and Rafiński (2006) were resurveyed. *Rana dalmatina* breeding sites are distributed in four clusters (Figs. 1–2) associated with the local extent of forest cover. Cluster I comprises 32 breeding sites over an extensive forested area west of the city of Tarnów and east of the Raba River. Cluster II, between the cities of Dębica and Tarnów, consists of 17 breeding sites. We found six breeding sites near the city of Dębica, in Cluster III. The straight line distances between neighboring clusters in southeastern Poland are approximately 17 (cluster I and II) and 11 km (cluster II

and III). In southeastern Poland we found five breeding sites close to the San River in the Sanok-Turka Range (cluster IV, Fig. 1).

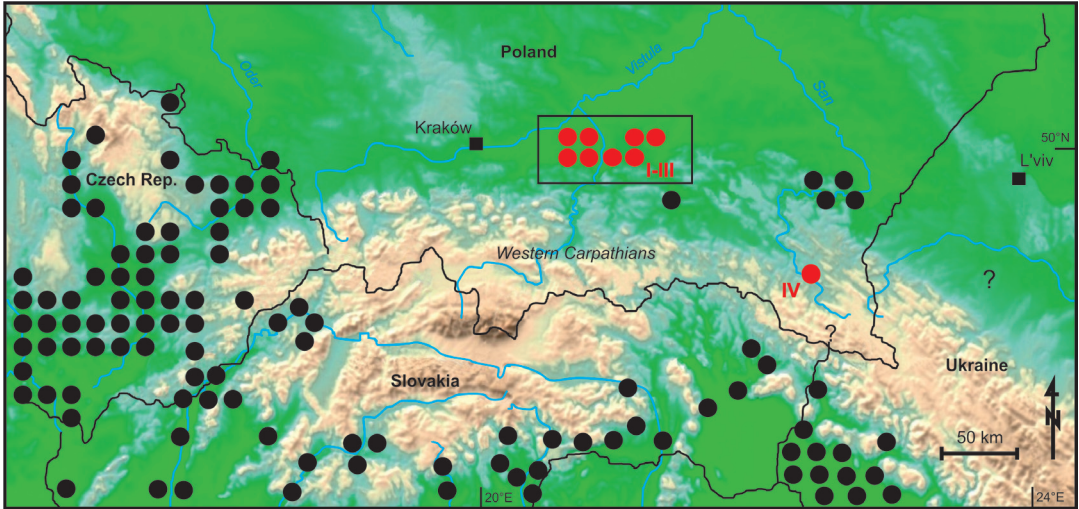
At some of the breeding sites we collected information on the number of egg clutches and habitat characteristics at the time of survey (Table 2). Less than ten clutches were counted at 26 sites, 17 breeding sites held between 20–100 clutches, and at four sites clutches numbered in the low hundreds. Although by no means representative of true census sizes of adult frogs, the number of clutches can give a rough indication of the number of breeding females at a site and therefore some idea on the abundance of frogs in a given area. Aquatic breeding habitat of *R. dalmatina* in southern Poland spans a range of man-made and natural water bodies including small ponds, sand, clay or peat pits (after excavation), irrigation ditches, small natural depressions and oxbows, and beaver ponds (Table 2). These breeding sites are typically found within or at the border of mixed woodland, and sometimes are found within villages.

#### Results of sequence analysis

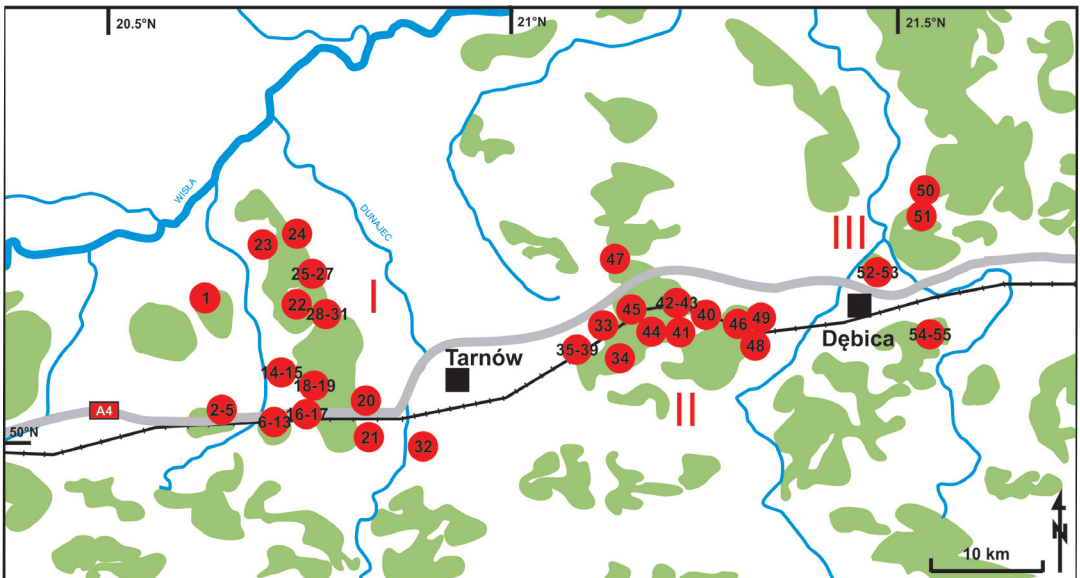
We used mitochondrial DNA sequence variation to confirm the identity of a subset of egg clutches found in the field. Our species diagnosis based on egg morphology was fully supported by the mtDNA data (Fig. 4). We obtained sequences of 710 bp for 13 brown frogs; all sequences have been deposited in GenBank under accession numbers JX481918–JX481930). Eggs and adults of the three brown frog species occurring sympatrically in southern Poland (*R. dalmatina*, *R. temporaria* and *R. arvalis*) can easily be distinguished at the molecular level by high divergence in their mtDNA. A total of 78 (11.3%) and 85 (12.3%) substitutions separate *R. dalmatina* from sympatric *R. arvalis* and *R. temporaria*, respectively. *Rana temporaria* and *R. arvalis* differ by 80 substitutions (11.6%). There was no sequence variation within 11 *R. dalmatina* individuals sampled from 11 localities in southern Poland (Fig. 4).

## Discussion

We describe 54 new breeding sites for *R. dalmatina* in southeastern Poland. This brings the total number of documented breeding sites in Poland (Szymura and Rafiński, 1997; Bartoń and Rafiński, 2006; Starzyk and Durak, 2007; Najbar, Vlček and Šuhaj, 2011) to over 76. However, this number is certainly an underestimate, as our study and previous surveys have not attempted to localize all potential breeding habitats in a given



**Figure 1.** The northeastern distribution of *Rana dalmatina*. Filled black circles indicate published occurrence data for *R. dalmatina* (Taraščuk, 1959; Lác, 1968; Moravec, 1994; Zavadil, 1997; Szymura, 2003; Reminnyi, 2007; Starzyk and Durak, 2007; Pisanets and Reminnyi, 2008; Najbar, Vlček and Šuhaj, 2011). Filled red circles indicate new localities described in this study. Roman numerals I-III (boxed insert) refer to groups of localities depicted in detail in Fig. 2, some of these datapoints overlap with localities provided by Szymura (2003); group IV refers to 5 new localities on the San River in southeastern Poland. Question marks (?) refer to unverified localities in southeastern Poland (Kowalski, 1970) and western Ukraine (Taraščuk, 1959).



**Figure 2.** Detailed distribution of *Rana dalmatina* in southeastern Poland (boxed insert in Figure 1). Roman numerals I-III refer to breeding sites in clusters I, II and III. The gray line refers to a major highway (A4) that is currently under construction. The black line represents a railroad along which many breeding sites were found and that could potentially facilitate dispersal for this species because of its associated man-made pools and ditches used as breeding habitat.



area. Moreover, earlier investigations reported juvenile agile frogs (e.g. Błachuta and Jabłoński, 1986; Rafiński, Szymura and Smykla, 1987; Szymura, 1994), but did not give information on breeding sites. *Rana dalmatina* has been previously reported from the Sandomierz Basin based on records of adults (Szymura, 1994, 2003) and a more detailed survey that provided some information on breeding and terrestrial habitat in the general area of cluster I (Bartoń and Rafiński, 2006). Our field work has revealed that this species is more extensively distributed and quite common in this region. The largest forest fragments south of the Vistula River probably hold the greatest amount of potential habitat and therefore the most numerous frog populations. Cluster I in particular, with 18 breeding ponds reported by Bartoń and Rafiński (2006) and a further 26 breeding sites reported herein, may rank as important at the regional scale due to the large number of man-made and natural water bodies and relatively extensive woodland habitat. Indeed, only six of the 54 breeding sites that we report were located further than 400 m from the closest woodland (Table 2). *Rana dalmatina* is nearly always associated with deciduous or mixed forest, and usually chooses breeding sites within or adjacent to woodland (Grossenbacher, 1997; Kuhn et al., 1997; Zavadil, 1997; Ficetola, Padoa-Schioppa and de Bernardi, 2008; Hartel et al., 2009). We counted >100 clutches of *R. dalmatina* eggs at three different breeding sites in cluster I, showing that this area could potentially support a large population of frogs. Clusters II and III also represent relatively extensive tracts of beech and pine forest, respectively, and may likewise contain large numbers of potential breeding sites, but are in need of detailed surveys.

Suitable forest habitat in the southern part of the Sandomierz Basin is, however, heavily fragmented at present. Apart from the largest forest fragments described above and a few sites located in small woodland patches (e.g. sites 1-5 and 32 in Fig. 2) most of the remaining deforested area is either intensively farmed or urbanized, and therefore most likely inhospitable for *R. dalmatina*. Population connectivity may thus be limited in this area. Terrestrial movements up to 300–500 m are known for individuals of this species during breeding migrations (Ponsero and Joly, 1998; Stümpel and Grosse, 2005). Moreover, agile frogs are known to colonize new ponds at distances up to 4 km from the nearest breeding site (Ahlén, 1997; Riis, 1997) and have been found up to 7 km from water bodies (Lác, 1959). We calculated mean distances between breeding sites within our most densely sampled areas. On average, breeding sites

were separated by  $8.1 \pm 4.7$  km (range: 0.2 - 21.5 km) and  $8.2 \pm 4.9$  km (range: 0.34 - 18.5 km) for clusters I and II, respectively. In wet years, however, flooded areas and irrigation ditches may provide for even greater density of breeding habitat. These estimates suggest that individual frogs may be able to regularly move within the suitable habitat afforded by unbroken woodland cover within each larger forest fragment and colonize or augment existing breeding populations, providing the foundation for metapopulation structure at the local scale. However, straight line distances  $\geq 11$  km across unsuitable habitat between adjacent breeding site clusters in the Sandomierz Basin suggest rather low levels of population connectivity at the regional scale. One possible avenue of dispersal among local agile frog populations could be via a railroad because of its associated man-made pools and ditches used as breeding habitat by this species (shown in Fig. 2). Apart from



**Figure 3.** Examples of typical *Rana dalmatina* breeding habitat in southern Poland: a natural small pond in deciduous woodland (top) and a man-made pool in mixed forest with 3 clutches of *R. dalmatina* eggs colonized by algae (bottom). The insert in the lower right shows an algae-colonized clutch of *R. dalmatina* eggs.

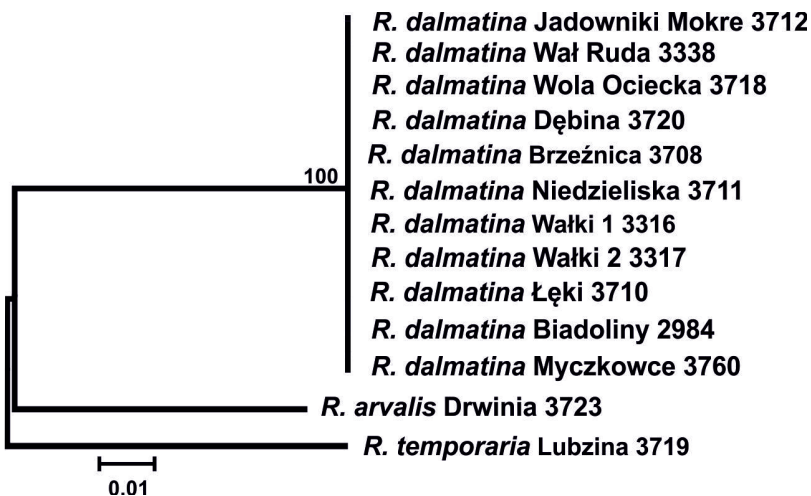
deforested land between *R. dalmatina* populations in the Sandomierz Basin, an additional threat that will likely lead to further habitat fragmentation is the construction of a major highway (labeled A4 in Fig. 2) currently underway. This species seems particularly susceptible to the road effect as demonstrated by increased mortality, diminished levels of genetic variation within populations, and greater differentiation between populations located close to major roads (Lesbarrères *et al.*, 2006). In addition, the construction of tunnels underneath highways as dispersal avenues is not very effective for this species (Lesbarrères *et al.*, 2004).

The five new localities in the Sanok-Turka Range (cluster IV) represent a hitherto unknown extension of the range of *R. dalmatina*. Breeding site 60 (Table 1) seems particularly significant due to many closely situated, natural water bodies (beaver ponds, small oxbows and depressions temporarily filled with water) that probably support a large breeding population of this species, as suggested by a one-time count of ca. 250 egg clutches (Table 2). Importantly, this site is protected as part of a natural reserve created for beavers.

#### Regional distribution

Our literature survey of the distribution of *R. dalmatina* at the northeastern margin of its range, despite the uneven survey effort in the different political jurisdictions, shows some general patterns. First, it is clear that *R. dalmatina* inhabits lowlands and river valleys penetrating into the Carpathian Mts. at its

northeastern distribution limits (Szymura and Rafiński, 1997). In Central Europe, very few localities occur above 500 m asl; most occur between 200 and 300 m asl (Guyétant, 1997; Zavadil, 1997; this study). Apart from valleys, this species is also present on hilly slopes fringing various mountain chains of the Carpathian (e.g. cluster IV in Fig. 1) and Sudetes massifs. Its distribution follows, sometimes quite far upstream, major tributaries of the Danube such as the Morava River in the Czech Republic, Váh and Hornád rivers in Slovakia, and the upper reaches of the Tisza River in western Ukraine. North of the Carpathians and Sudetes, *R. dalmatina* is present in the headwaters of the Oder River drainage system, as confirmed by many localities in the Czech Republic (Zavadil, 1997) and a recent record from southern Poland (Najbar, Vlček and Šuhaj, 2011). This region is of particular interest since it encompasses the Moravian Gate, a depression between the Carpathian Mts. in the east and the Sudetes in the west, and may possibly constitute a dispersal corridor for this species. In southeastern Poland, *R. dalmatina* is present in the Sandomierz Basin (clusters I-III) and in the foothills of the Carpathian Mts. about 40 km south of cluster III (Starzyk and Durak, 2007). This species also occurs in southeastern Poland along the San River, from the vicinities of Przemyśl in the north (Rafiński, Szymura and Smykla, 1987), to the Sanok-Turka hills located on the northern border of the Carpathians (cluster IV). In Ukraine, *R. dalmatina* occurs in the Zakarpattia Province west of the main ridge of the Eastern Carpathians, and



**Figure 4.** Phylogram showing the relationships between three brown frog species (*Rana arvalis*, *R. dalmatina* and *R. temporaria*) sampled from southern Poland, based on a 710 bp fragment of the ND1 gene. Neighbor-joining tree with bootstrap support. Localities and sample numbers are given after the species name.

**Table 1.** Geographical coordinates of *Rana dalmatina* breeding sites in southern Poland. Cluster refers to clusters I-IV in Figs 1-2. Numbers in the second column (Site) give the approximate locations of each site in Fig. 2. Asterisks (\*) denote localities surveyed previously by Bartoń and Rafiński (2006).

Cluster	Site	Locality	Geographical coordinates		Cluster	Site	Locality	Geographical coordinates	
I	1	Niedzieliska	50.091396°N	20.645406°E	I	31	Waryś	50.069749°N	20.772168°E
I	2	Sterkowiec	49.991482°N	20.670867°E	I	32	Kobiela	49.98495°N	20.89626°E
I	3	Wokowice	50.0072 °N	20.69678° E	II	33	Wałki	50.060278°N	21.151667°E
I	4	Lęki	50.017802°N	20.681386°E	II	34	Wielkie Pole	50.040590°N	21.166105°E
I	5	Szczepanów	50.01353°N	20.65931°E	II	35	Wola Rzędzińska	50.035380°N	21.069614°E
I	6	Dębno	49.985836°N	20.722633°E	II	36	Wola Rzędzińska	50.043245°N	21.072479°E
I	7	Biadolino	49.999014°N	20.719564°E	II	37	Wola Rzędzińska	50.046466°N	21.06585°E
I	8	Biadolino*	49.996240°N	20.720988°E	II	38	Wola Rzędzińska	50.049083°N	21.07055°E
I	9	Biadolino*	49.990214°N	20.723185°E	II	39	Wola Rzędzińska	50.048983°N	21.075266°E
I	10	Biadolino*	50.003141°N	20.734373°E	II	40	Czarna	50.058056°N	21.265556°E
I	11	Biadolino	50.006890°N	20.735004°E	II	41	Czarna	50.052500°N	21.230278°E
I	12	Biadolino	50.00295°N	20.718283°E	II	42	Czarna	50.066667°N	21.223056°E
I	13	Biadolino	50.0012°N	20.7185°E	II	43	Czarna	50.063889°N	21.202222°E
I	14	Bielcza	50.009266°N	20.738721°E	II	44	Czarna	50.052222°N	21.204722°E
I	15	Bielcza	50.031846°N	20.731771°E	II	45	Czarna	50.065833°N	21.183333°E
I	16	Biadolino*	50.006948°N	20.745197°E	II	46	Grabiny	50.048889°N	21.305833°E
I	17	Biadolino*	50.004597°N	20.757163°E	II	47	Nowa Jastrzębka	50.126931°N	21.145509°E
I	18	Bielcza*	50.023563°N	20.760201°E	II	48	Brzeziny	50.041083°N	21.2299°E
I	19	Bielcza	50.017220°N	20.753292°E	II	49	Głowaczowa	50.06025°N	21.324483°E
I	20	Łętowice	50.007168°N	20.831256°E	III	50	Wola Ociecka	50.156649°N	21.541732°E
I	21	Dębina Zakrzowska	49.972540°N	20.828577°E	III	51	Budy	50.135655°N	21.539660°E
I	22	Borzęcin	50.084126°N	20.759233°E	III	52	Brzeźnica near Dębica	50.087220°N	21.479865°E
I	23	Dolega	50.127976°N	20.721124°E	III	53	Pustynia	50.080501°N	21.472420°E
I	24	Jadowniki Mokre	50.139525°N	20.757293°E	III	54	Lubzina	50.0559°N	21.52321°E
I	25	Wał Ruda	50.104682°N	20.777991°E	III	55	Stobierna	50.038°N	21.483611°E
I	26	Wał Ruda	50.102660°N	20.781929°E	IV	56	Myczkowce	49.446389°N	22.410833°E
I	27	Wał Ruda	50.119732°N	20.776155°E	IV	57	Myczkowce	49.431667°N	22.403889°E
I	28	Brzeźnica near Radłów	50.078178°N	20.794162°E	IV	58	Myczkowce	49.445278°N	22.398611°E
I	29	Brzeźnica near Radłów	50.078900°N	20.822746°E	IV	59	Myczkowce	49.442833°N	22.405361°E
I	30	Brzeźnica near Radłów	50.071856°N	20.823878°E	IV	60	Uherce Mineralne	49.456450°N	22.394917°E

the territories surrounding Moldova, including directly north in the region of Chernivtsi (Ščerbak and Ščerban<sup>7</sup>, 1980; Reminnyi, 2007; Pisanets and Reminnyi, 2008). The current understanding is that the eastern range of this species does not seem to cross the Dniestr River. At present there are no confirmed localities from northwestern Ukraine east of the Carpathians, although Tarašcuk (1959) mentions a locality in the vicinities of Morshyn, that is, however, in need of confirmation. In summary, *R. dalmatina* inhabits lowlands, river valleys and foothills surrounding the Western Carpathian Mts. which probably constitute a significant barrier for the dispersal of this species.

The lack of mtDNA sequence variation in individuals from 11 populations of *R. dalmatina* studied herein suggests that the colonization of southern Poland occurred recently and probably from a single source population (e.g. a southern refugium). Assuming that

the Western Carpathians have obstructed the northward dispersal of *R. dalmatina* in this region since the Pleistocene, three mutually non-exclusive hypotheses can be put forward to explain the presence of this species in southern Poland. *R. dalmatina* could have colonized in an easterly direction from a formerly more extensive distribution in western Poland and eastern Germany, as has been demonstrated for the alpine newt (Pabijan and Babik, 2006). On the other hand, the Carpathians could have been flanked from the east along Dniestr River in Ukraine, reaching southern Poland via a westward extension of this expansion. Because some of our sites are located very close to the Ukrainian border, it is highly probable that unrecorded populations of this species inhabit northwestern Ukraine, and we flag this area as a priority for future surveys. Finally, populations in southern Poland could have originated from a northward expansion through the Moravian Gate along

**Table 2.** Habitat characteristics of 47 *R. dalmatina* breeding sites in southern Poland. The first column gives the number of the locality (coordinates in Table 1). No. clutches refers to the approximate number of egg clutches counted at a breeding site. If a breeding site was visited several times, then the highest clutch census is given. No. ponds gives the number of ponds at a locality (i.e. some breeding sites consist of adjacent small water bodies). Distance refers to the distance (in meters) to the nearest forest patch (a forest patch is defined as >1 ha of tree cover); a distance of zero means that the locality lies within forest or woodland. Aquatic habitat gives a concise description of the water body in which *R. dalmatina* eggs were found and approximate water surface area at the time of survey, if available. The last column provides a description of the terrestrial habitat in the direct surroundings of the breeding site.

Site	No. clutches	No. ponds	Distance	Aquatic habitat (area)	terrestrial habitat
1	<10	2	0	sand pits (200-800 m <sup>2</sup> )	pine forest, shrubs, crops,
2	~300	14	0	beaver ponds, depressions, ditches (~2200 m <sup>2</sup> )	mixed forest, meadows, railroad
4	<10	1	0	sand pit	alder forest, crops
6	~20	1	0	small pond (~9 m <sup>2</sup> )	mixed forest, rubbish disposal
7	<10	1	0	small pond (~30 m <sup>2</sup> )	mixed forest
8	<10	1	0	small pond (~30 m <sup>2</sup> )	mixed forest
9	~100	1	0	shallow depression (~400 m <sup>2</sup> )	mixed forest (alder/pine)
10	<10	1	10	ditch	village
11	<10	2	420	small ponds (~4 m <sup>2</sup> )	crops, rubbish disposal
13	~20	2	0	small ponds (25 m <sup>2</sup> and 64 m <sup>2</sup> )	mixed forest, meadow
14	<10	2	240	oxbows	meadows, crops, shrubs
15	~40	1	400	ditch and flooded meadow	meadows
16	5	1	90	desiccating oxbow (500 m <sup>2</sup> )	meadows, crops, shrubs
17	~50	2	260	ditch near railroad	shrubs, village
18	~100	2	100	peat pond	meadows, shrubs
19	~30	1	0	sand pit (up to 2,000 m <sup>2</sup> depending on water level)	pine forest
20	~30	2	0	depression (2,500 m <sup>2</sup> ), sand pit (6 m <sup>2</sup> )	mixed forest, crops, railroad
21	<10	1	290	small pond	meadows, shrubs, village, crops
22	~30	2	260	large and deep sand pits (10,000 m <sup>2</sup> and 2,000m <sup>2</sup> )	meadows, shrubs
23	~30	8	210	complex of ponds and ditches (500-600 m <sup>2</sup> )	shrubs, village
24	~10	1	160	sand pit (600 m <sup>2</sup> )	shrubs, meadows, crops
25	~20	1	0	sand pit (3400 m <sup>2</sup> )	mixed forest, meadows
26	~50	1	10	large flooded depression (3,700 m <sup>2</sup> )	meadows, shrubs
27	~80	3	130	small ponds (70-300 m <sup>2</sup> )	meadow, crops, shrubs
28	~20	2	0	small ponds (70 m <sup>2</sup> and 150 m <sup>2</sup> )	mixed forest, meadow
29	<10	2	0	small pond (10 m <sup>2</sup> )	mixed forest, meadows, shrubs
30	~30	2	380	sand pits (1,700 m <sup>2</sup> and 6,500 m <sup>2</sup> )	mixed forest, meadows
31	<10	1	0	ditch	mixed woodland
35	<10	1	1,000	small pond (100 m <sup>2</sup> )	village, crops, shrubs
36	~50	3	1,200	ditch, clay excavation	crops, shrubs, meadows
37	3	1	1,600	small pond	shrubs, crops
38	4	3	1,200	small pond, ditch	shrubs, crops
39	9	1	800	ditch	shrubs, crops, grove of trees
46	10-100	?	60	complex of peat and sand pits	alder/pine forest, crops, village
47	<10	1	150	small pond (25 m <sup>2</sup> )	shrubs, meadows, crops, village
48	~40	3	0	3 small ponds	pine forest, meadow
49	2	1	30	pond (200 m <sup>2</sup> )	pine forest, meadow
50	~10	3	350	sand pits (20-100 m <sup>2</sup> )	shrubs, meadows, crops
51	<10	1	0	ditch (250 m <sup>2</sup> )	mixed forest, crops, village
52	~60	2	0	large, connected ponds (2,700 m <sup>2</sup> and 20,000 m <sup>2</sup> )	mixed forest, meadows, crops
53	~10	1	50	ditch	shrubs, mixed forest
54	<10	1	0	wheel rut (3 m <sup>2</sup> )	mixed forest
55	<10	1	0	ditch (15 m <sup>2</sup> )	meadow, mixed forest
56	<10	39	0	puddles on road	mixed forest
57	<10	2	0	ponds	mixed forest
58	<10	2	600	small artificial ponds (4 m <sup>2</sup> )	park and village
60	~250	28	0	small and large beaver ponds, small oxbows	alder forest



Oder River, an area from which contemporary localities are known. Detailed surveys are required along the upper Vistula and the Oder rivers, as these river valleys could have acted as avenues of dispersal for the species. The contributions of each of the three colonization scenarios outlined above could be elucidated through the application of quickly evolving molecular markers such as microsatellites.

## References

- Ahlén, I. (1997): Distribution and habitats of *Rana dalmatina* in Sweden. *Rana* **2**: 13–22.
- Araújo, M.B., Thuiller, W., Pearson, R.G. (2006): Climate warming and the decline of amphibians and reptiles in Europe. *J. Biogeogr.* **33**: 1712–1728.
- Bartoń, K., Rafiński, J. (2006): Co-occurrence of agile frog (*Rana dalmatina* Fitz. in Bonaparte) with common frog (*Rana temporaria* L.) in breeding sites in southern Poland. *Polish J. Ecol.* **54**: 151–157.
- Baumgartner, C., Bitschi, N., Ellinger, N., Gollmann, B., Gollmann, G., Köck, M., Lebeth, E., Waringer-Löschenkohl, A. (1996): Laichablage und Embryonalentwicklung von Springfrosch (*Rana dalmatina* Bonaparte, 1840) und Grasfrosch (*Rana temporaria* Linnaeus, 1758) in einem syntopen Vorkommen. *Herpetozoa* **9**: 133–150.
- Blachuta, J., Jabłoński, A. (1986): Nowe stanowisko żaby dalmatyńskiej (*Rana dalmatina*, Bonaparte) w Polsce. *Prz. Zool.* **30**: 207–212.
- Bonk, M., Pabijan M. (2010): Changes in a regional batrachofauna in south-central Poland over a 25 year period. *North-West J. Zool.* **6**: 225–244.
- Borkin, L.J., (1977): Czy w Polsce występuje żaba dalmatyńska, *Rana dalmatina* Bonaparte? *Prz. Zool.* **30**: 40–44.
- Ficetola, G.F., De Bernardi, F. (2005): Supplementation or in situ conservation? Evidence of local adaptation in the Italian agile frog *Rana latastei* and consequences for the management of populations. *Anim. Conserv.* **8**: 33–40.
- Ficetola, G.F., Padoa-Schioppa, E., de Bernardi, F. (2008): Influence of landscape elements in riparian buffers on the conservation of semiaquatic amphibians. *Conserv. Biol.* **23**: 114–123.
- Gasc, J.-P., Cabela, A., Crnobrnja-Isailovic, J., Dolmen, D., Grossenbacher, K., Haffner P., Lescure, J., Martens, H., Martínez Rica, J. P., Maurin, H., Oliveira, M. E., Sofianidou, T. S., Veith, M., Zuidervijk, A. (1997): Atlas of amphibians and reptiles in Europe. Societas Europaea Herpetologica & Muséum National d'Histoire Naturelle, Paris.
- Gibson, S.Y., van der Marel, R.C., Starzomski, B.M. (2009): Climate change and conservation of leading-edge peripheral populations. *Conserv. Biol.* **23**: 1369–1373.
- Grossenbacher, K. (1997): The agile frog *Rana dalmatina* in Switzerland. *Rana* **2**: 59–65.
- Guyétant, R. (1997): Distribution and characteristics of the biology of the agile frog (*Rana dalmatina* Bonaparte) in France. *Rana* **2**: 39–43.
- Hamilton, J.A., Eckert, C.G. (2007): Population genetic consequences of geographic disjunction: a prairie plant isolated on Great Lakes alvars. *Mol. Ecol.* **16**: 1647–1660.
- Hartel, T., Szilárd, N., Cogălniceanu, D., Öllerer, K., Moga, C.I., Lesbarrères, D., Demeter, L. (2009): Pond and landscape determinants of *Rana dalmatina* population sizes in a Romanian rural landscape. *Acta Oecol.* **35**: 53–59.
- Juszczyk, W. (1987): *Plazy i gady krajowe*. Warszawa, PWN.
- Kowalski, W. (1970): Żaba zwinka, *Rana dalmatina* Bonap. 1840, w Polsce. *Prz. Zool.* **14**: 310–308.
- Kuhn, J., Gnath-Austen, F., Gruber, H.-J., Krach, E., Reichhlof, J.H., Schäffler, B. (1997): Distribution, habitats, and population vulnerability of the agile frog (*Rana dalmatina*) in Bavaria. *Rana* **2**: 127–142.
- Lác, J. (1959): Verbreitung des Springfrosch (*Rana dalmatina* Bon.) in der Slowakei und Bemerkungen zu deren Bionomie. *Biologia, Bratislava* **14**: 117–134.
- Lác, J. 1968: Oboživelníky – Amphibia. In: Stavovce. Slovenska I – Ryby, oboživelníky a plazy (eds. O. Oliva., S. Hrabě and J. Lác). Vyd. SAV, Bratislava.
- Lesbarrères, D., Lodé, T., Merilä, J. (2004): What type of amphibian tunnel could reduce roadkills? *Oryx* **38**: 220–223.
- Lesbarrères, D., Primmer, C.R., Lodé, T., Merilä, J. (2006): The effects of 20 years of highway presence on the genetic structure of *Rana dalmatina* populations. *Ecoscience* **13**: 531–538.
- Moravec, J. (1994): Atlas rozšíření oboživelníků v České republice. Atlas of Czech amphibians. Národní muzeum, Praha.
- Najbar, B., Vlček, P., Šuhaj, J. (2011): New locality record for the agile frog (*Rana dalmatina*) from and Odra River meander in southern Poland. *Herp. Notes* **4**: 063–065.
- Pabijan, M. (2009): Traszka grzebieniasta *Triturus cristatus* (Laurenti, 1768). In: *Metodyka monitoringu- przewodniki metodyczne*, pp 195–219. Główny Inspektorat Ochrony Środowiska, Warszawa.
- Pabijan, M., Babik, W. (2006): Genetic structure in northeastern populations of the Alpine newt *Triturus alpestris*: evidence for post-Pleistocene differentiation. *Mol. Ecol.* **15**: 2397–2407.
- Parnesan, C. (2006): Ecological and evolutionary responses to recent climate change. *Annu. Rev. Ecol. Evol. Syst.* **37**: 637–669.
- Pisanets, E.M., Reminnyi, V.Yu. (2008): New data about distribution of Agile Frog, *Rana dalmatina* (Amphibia, Anura, Ranidae), in Ukraine and it Eastern Border of Natural Habitat. *Pratsi Ukrayinskogo Gerpetologichnogo Tovarystva*, **1**: 77–86.
- Ponsero, A., Joly, P. (1998): Clutch size, egg survival and migration distance in the agile frog (*Rana dalmatina*) in a floodplain. *Arch. Hydrobiol.* **12**: 343–352.
- Rafiński J., Szymura J.M. (1991): Neue Fundorte des Springfrosches *Rana dalmatina* (Bonaparte 1940) aus Südspolen. In: *Amphibienforschung und Vivarium*, p. 135–137. Hermann, H.J., Ed.
- Rafiński J., Szymura J.M. (2001): *Rana dalmatina* (Bonaparte, 1840) Agile frog. In: *Polish Red Data Book of Animals. Vertebrates*, p. 287–288. Głowaciński Z. (red.). Państwowe Wydawnictwo Rolnicze i Leśne, Warszawa.

- Rafiński, J., Szymura, J.M., Smykla, J. (1987): Elektroforetyczna identyfikacja żaby zwinki (*Rana dalmatina* Bonaparte) z nowych stanowisk w południowo-wschodniej Polsce. *Prz. Zool.* **31**: 493–502.
- Reminnyi, V.Yu. (2007): New data about the eastern border of natural habitat of Agile Frog *Rana dalmatina* (Ranidae, Amphibia). *Sci. Bull. Uzhgorod Univ. (Ser. Biol.)*, **21**: 113–116.
- Riis, N. (1997): Field studies on the ecology of the agile frog in Denmark. *Rana* **2**: 189–202.
- Stümpel, N., Grosse, W.-R. (2005): Phänologie, Aktivität und Wachstum von Springfröschen (*Rana dalmatina*) in unterschiedlichen Sommerlebensräumen in Südostniedersachsen. *Z. Feldherpetol.* **12**: 71–99.
- Ščerbak, N.N., Ščerban', M.I. (1980): *Zemnovodne i Premykajuščiesja Ukrainskich Karpat*. Naukova Dumka, Kiev.
- Starzyk, N., Durak, R. (2007): New localities of the Dalmatian frog *Rana dalmatina* Bonaparte in south-eastern Poland. *Prz. Zool.* **51**: 51–55.
- Szymura, J.M. (1994): The agile frog, *Rana dalmatina* Bonaparte, near Biadoliny in southern Poland. *Prz. Zool.* **28**: 93–95.
- Szymura, J.M. (2003): Żaba zwinka *Rana dalmatina* Bonaparte, 1840. In (Głowaciński, Z., Rafiński, J. (eds.) *Atlas of amphibians and reptiles of Poland. Status – distribution – conservation*. Biblioteka Monitoringu Środowiska, Warszawa – Kraków.
- Szymura, J.M., Rafiński, J. (1997): The agile frog *Rana dalmatina* Bonaparte, 1840 in Poland. *Rana* **2**: 35–37.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S. (2011): MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Mol. Biol. Evol.* **28**: 2731–2739.
- Tarašcuk, W.I. (1959): *Fauna Ukrainy*, **7**, *Zemnovodni ta plazunni*. Kyiv, Vidavnictvo Akademii Nauk Ukrain's'koi RSR.
- Williams, J.W., Jackson, S.T., Kutzbach, J.E. (2007): Projected distributions of novel and disappearing climates by 2100 A.D. *Proc. Nat. Acad. Sci. USA* **104**: 5738–5742.
- Zavadil, V. (1997): Zur Verbreitung, Biologie und zum Status des *Rana dalmatina* in der Tschechischen Republik mit Anmerkungen zur Bionomie aus der Slowakei. *Rana* **2**: 45–58.