

# Population systems of the *Pelophylax esculentus* complex in the southern part of its range

Imre I. KRIZMANIĆ<sup>1\*</sup> and Ana T. IVANOVIĆ<sup>1</sup>

<sup>1</sup> Institute of Zoology, Faculty of Biology, Studentski trg 16, 11000 Belgrade, Serbia;  
e-mail: krizmanic.imre@bio.bg.ac.rs, ana@bio.bg.ac.rs

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**Abstract.** We explored the population systems of *Pelophylax esculentus* at the southern border of the distribution range for this complex. We used both morphological data and allozyme markers to describe population systems in this region. All three taxa in the complex, *P. ridibundus*, *P. lessonae* and their hybridogenetic form, *P. kl. esculentus* occurred in the research area. In four localities that were analysed near the Danube River, we found complex population systems of both the parent species and their hybridogenetic form. Southward from the border defined by the Sava and Danube riverbanks, only the *P. ridibundus* – *P. kl. esculentus* (R-E) system was detected. Among these R-E populations, the abundance of the hybridogenetic form declines sharply from the north to the south. The most common and widespread system in Central Europe, the *P. lessonae* – *P. kl. esculentus* population system, was detected in only one locality near the Sava River.

**Key words:** *Pelophylax ridibundus*, *P. lessonae*, *P. kl. esculentus*, distribution, Serbia

## Introduction

*Pelophylax kl. esculentus* is a widespread natural hybrid that is produced as offspring of the parent species *P. lessonae* (Camerano, 1882) and *P. ridibundus* (Pallas, 1771). The hybrids can reproduce by hybridogenesis, in which the set of chromosomes that is derived from one parental species is completely discarded, while the set from the other parental species undergoes compensatory duplication. *Pelophylax kl. esculentus* gametes thus contain an unrecombined genome derived from one parental species (Hotz et al. 1985, Graf & Polls-Pelaz 1989, Berger 1990, Tunner & Hepich Tunner 1992, Raghianti et al. 2007).

The geographical patterns and population systems composition of the western Palearctic water frogs complex (*P. esculentus* complex) have been well-studied over a significant part of their distribution range, especially in areas where all three forms co-occur. Seven different systems have generally been described that differ in composition with respect to the presence of each taxon, their relative abundance and reproductive relationships (e.g. Graf & Polls-Pelaz 1989, Berger 1990, Plötner & Grunwald 1991, Plötner et al. 1994, Rybacki & Berger 2001, Borkin et al. 2002). Despite the considerable amount of

available data, our knowledge about the occurrence, distribution and structure of western Palearctic water frog population systems at the southern border of their distribution range remains very limited (Spasić Bošković et al. 1999). *Pelophylax ridibundus* is a widespread species throughout Serbia. However, the location and population structure of *P. lessonae* in this area is largely unknown. The scarce data that do exist on the distribution of *P. lessonae* are from the region north of the Danube and Sava Rivers (e.g., Karaman 1948). The available data for the hybrid taxa (*P. kl. esculentus*) are also mostly from the northern Pannonian and peri-Pannonian parts of Serbia (Radovanović 1951, Radovanović 1964, Spasić 1996, Spasić Bošković et al. 1999, Džukić et al. 2001, Krizmanić 2008).

The aim of this study was to examine the presence, geographical distribution patterns, and species composition of the western group of Palearctic water frogs at the southern border of their range, which is partially within the territory of Serbia. More specifically, we attempted to establish the southern distribution limit of *P. lessonae* in the peri-Pannonian part of Serbia along its major rivers.

## Material and Methods

We examined frog populations from 15 sites (Fig. 1). In total, 388 specimens were collected for morphometric analysis. For each frog, we measured ten traits: body length (L), femur length (F), tibia length (T), internasal distance (Spi), maximum head width (Ltc), length of the first toe of hind leg (DpPp), metatarsal tubercle length (Cint), minimal interorbital distance (Spp), snout-eye distance (Dro), diameter of the tympanic membrane (Lty). All measurements were performed by the same person (I.K.) using digital callipers (0.1 mm accuracy). To explore morphological variation among taxa, we analyzed 18 indices of external morphological

characteristics (L/F, L/T, L/Spi, L/Ltc, L/DpPp, L/Cint, L/Spp, L/Dro, L/Lty, F/T, F/DpPp, F/Cint, T/DpPp, T/Cint, DpPp/Cint, Spi/Spp, Ltc/Dro, and Ltc/Lc) using one-way ANOVA and discriminant analysis (Krizmanić 2008). For all statistical analyses, we used the statistical software package Statistica for Windows 6.0 (StatSoft 2001).

Electrophoretic variability was analysed for three loci in the total sample of 388 specimens and for one locus (PGDH) on a reduced sample of 357 specimens. Enzymes were evaluated on an 11.5% starch gel system using standard procedures (Murphy 1996). We analysed four enzyme loci: glycerol-3-phosphate

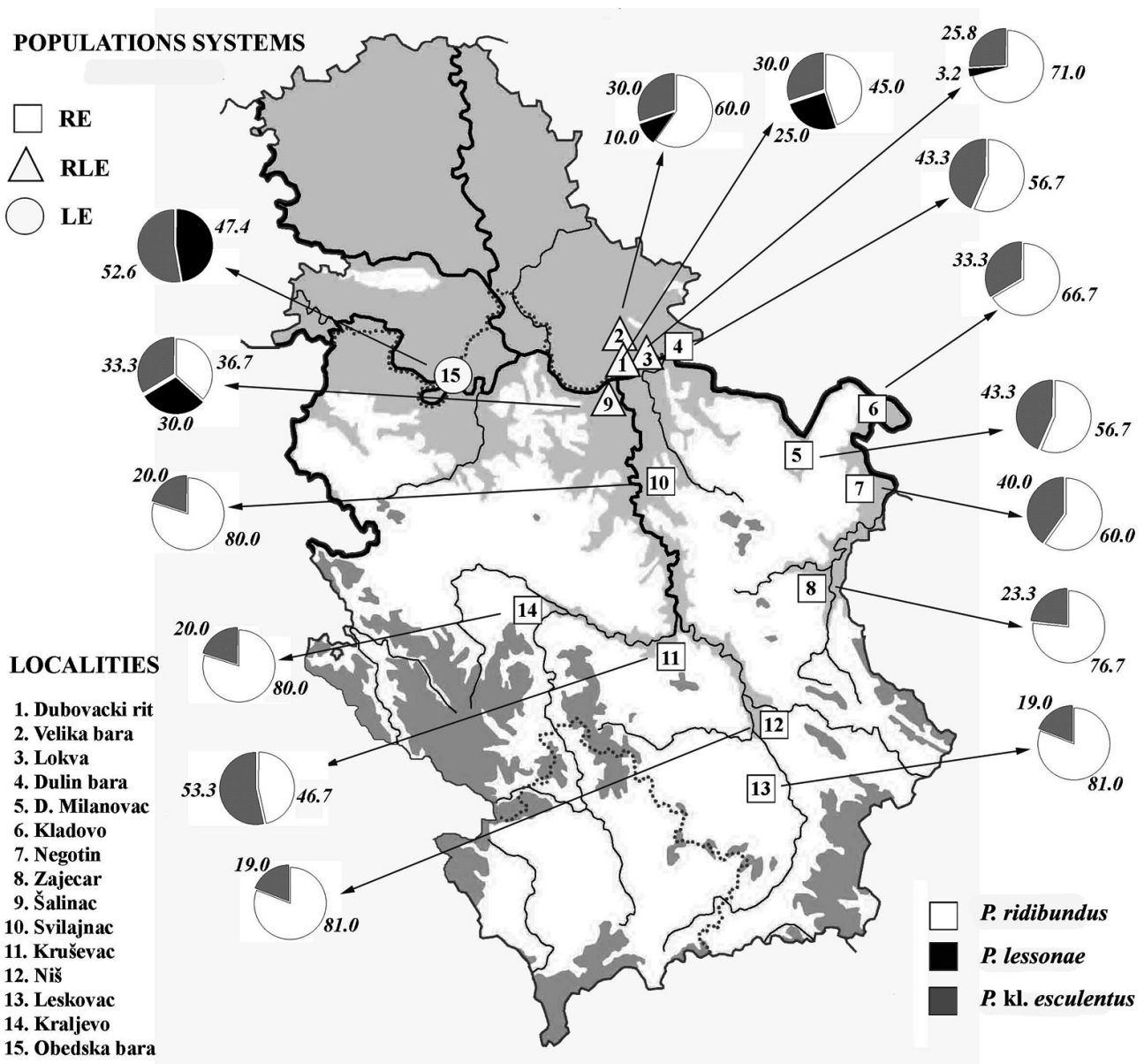


Fig. 1. Distribution of population systems in the southern part of the complex range, with the proportion of each registered taxa.

dehydrogenase (G3PDH EC 1.1.1.8), (Formerly:  $\alpha$  glycerophosphate dehydrogenase –  $\alpha$  GPD or  $\alpha$  GPDH); glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49); L – lactate dehydrogenase (LDH-1 EC 1.1.1.27); phosphogluconate dehydrogenase (PGDH, EC 1.1.1.44) (Formerly: 6-phosphogluconate dehydrogenase – 6 PGD or 6 PGDH). The nomenclature of enzymes and E. C. numbers followed Murphy et al. (1996). For all genetic analyses, we used the TFGPA 1.3 software package (Miller 1997). Accordingly, identification of taxa was accomplished by combining both the distinctive morphometric indices (Schneider et al. 1984, Günther et al. 1991, Pagano & Joly 1999, Lode & Pagano 2000) and the selected polymorphic loci (Günther et al. 1991, Beerli 1994, Kotlík 1996).

*List of localities with coordinates of latitude and longitude and numbers of investigated individuals*

1. Dubovački rit 44° 47.727' /21° 12.779' /20;
2. Velika bara 44° 49.712' /21° 18.921' /30;
3. Lokva 44° 50.365' /21° 18.404' /31;

4. Dulin bara 44° 51.220' /21° 18.299' /28;
5. Donji Milanovac 44° 27.995' /22° 09.405' /29;
6. Kladovo 44° 36.692' /22° 36.910' /30;
7. Negotin 44° 13.570' /22° 32.182' /28;
8. Zaječar 43° 47.190' /22° 18.818' /30;
9. Šalinci 44° 41.340' /21° 00.800' /30;
10. Svilajnac 44° 13.950' /21° 12.105' /23;
11. Kruševac 43° 39.390' /21° 23.390' /29;
12. Niš 43° 24.240' /21° 47.100' /30;
13. Leskovac 42° 52.970' /21° 53.425' /21;
14. Kraljevo 43° 47.770' /20° 35.620' /10;
15. Obedska Bara 44° 44.200' /19° 59.620' /19.

**Results**

Results of ANOVA demonstrated that morphometric ratios differed significantly among taxa (Table 1). The indices producing the highest degree of separation between the taxa were DpPp/Cint, T/Cint, L/T and F/T. Discriminate analyses based on the ANOVA results showed clear separation of the parental species (Fig. 2, Fig. 3). The range of variation of the morphological

**Table 1.** Descriptive characteristics and comparison of selected morphometric indices among examined taxa (SD, standard deviation; DpPp, length of the first toe of hind leg; Cint, metatarsal tubercle length; T, tibia length; L, body length; F, femur length).

Taxon		DpPp/Cint	T/Cint	L/T	F/T
<i>P. ridibundus</i>	min.	1.60	6.44	1.72	0.80
	max.	3.00	11.81	2.45	1.04
	mean	2.33	8.68	2.01	0.94
	SD	0.31	0.97	0.13	0.06
<i>P. kl. esculentus</i>	min.	1.23	5.16	1.96	0.97
	max.	1.90	8.10	2.67	1.17
	mean	1.64	6.47	2.25	1.03
	SD	0.04	0.79	0.17	0.05
<i>P. lessonae</i>	min.	1.60	6.44	1.72	0.80
	max.	3.02	11.80	2.45	1.04
	mean	2.33	8.68	2.01	0.94
	SD	0.32	0.97	0.13	0.06
ANOVA	F	110.59	109.53	91.17	53.42
	p	< 0.001	< 0.001	< 0.001	< 0.001

**Table 2.** Allozymic variation detected among the three water frog taxa.

Taxon	G3PDH	G6PDH	LDH	PGDH
<i>P. ridibundus</i>	a, rarely b	b, rarely a,c	a, rarely b,c	c,b, rarely a
<i>P. lessonae</i>	b	a, rarely b	c, rarely a,b	a, rarely b
<i>P. kl. esculentus</i>	a,b	a,b, rarely c	a,c, rarely b	a,b,c

features ratios in *P. kl. esculentus* populations was within the range of variation of both parent species (Fig. 3). The relative electrophoretic mobility of the allelic products was used for diagnostic purposes (Table 2). The fastest alleles (a) for both LDH and G3PDH were characteristic of individuals that were identified as *P. ridibundus* based on morphometry. The second allozyme phenotype was a marker for *P. lessonae* and included homozygotes for the G3PDH (b), LDH (c) and PGDH (a). All animals heterozygotic for G3PDH and LDH, were *P. kl. esculentus*. The next group included individuals heterozygous for LDH (a/c or b/c) and homozygous for G3PDH (b), and, significant differences in morphometry were not recognized between this group and animals identified as *P. lessonae*. *Pelophylax kl. esculentus* revealed a heterozygotic (a/b) phenotype for G6PDH and a heterozygotic (a/c or a/b) phenotype for PGDH. According to both morphological and genetic data, three population systems occurred in our study area:

*P. ridibundus* – *P. lessonae* – *P. kl. esculentus* (R-L-E), *P. lessonae* – *P. kl. esculentus* (L-E), and *P. ridibundus* – *P. kl. esculentus* (R-E) (Fig. 1). *Pelophylax ridibundus* was recorded almost throughout the entire research area and was the most abundant species of water frogs in the system as a whole (except for populations, No. 11 and 15). The second parental species (*P. lessonae*) was restricted to two systems, R-L-E and L-E. Interestingly, we did not find “pure” *ridibundus* populations. The L-E population system occurred in one locality only (Obedska bara, No. 15).

### Discussion

Our results indicate that the R-E-L system could be the most frequent system in the border area towards the east. *Pelophylax lessonae*, which was the least abundant species in our study population, propagates along major rivers throughout Serbia (especially the Sava and the Danube Rivers), and thus it seems likely that *P. lessonae* inhabits a much wider area (Colganiceanu & Tesio

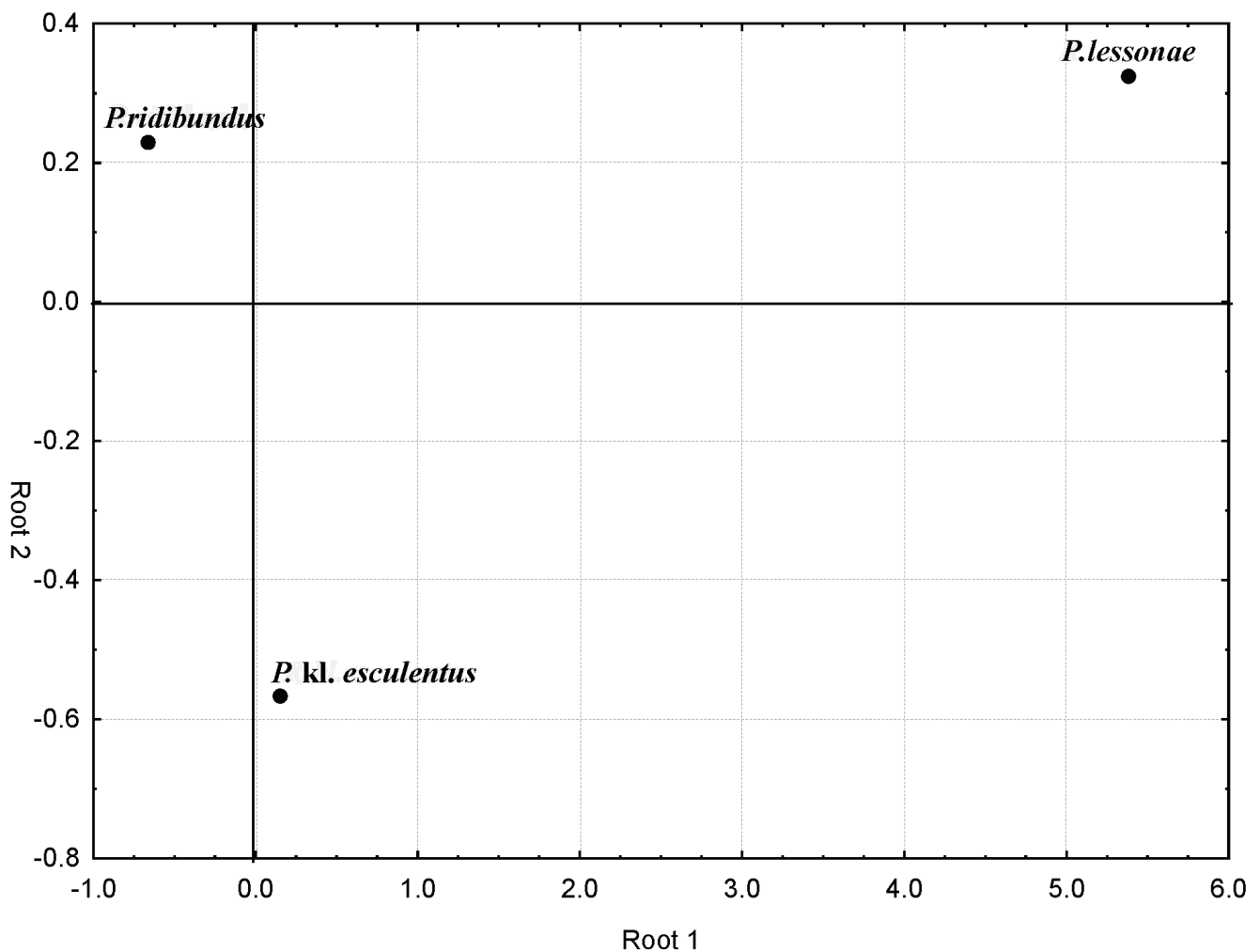
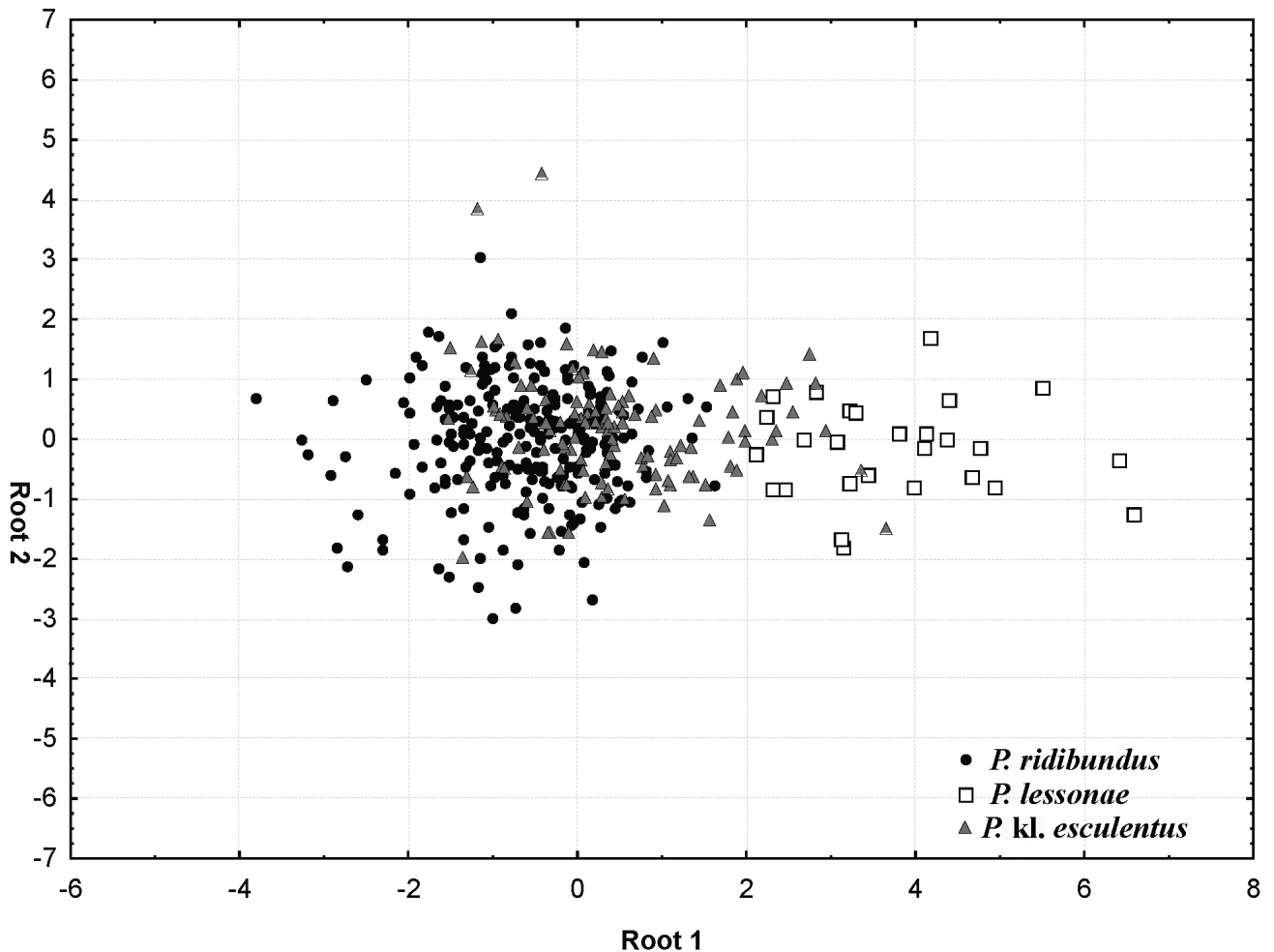


Fig. 2. Position of the group centroids in the projection of the first and the second canonical variants.



**Fig. 3.** Relative position of individuals in the projection of the first and the second canonical variants.

1993). The hybridogenetic species (*P. kl. esculentus*) occurred in all localities that we investigated with a level of relative abundance that varied between the localities. The R-E-L system is considered to be rare in Europe (Berger 1990, Lada et al. 1995, Rybacki & Berger 2001, Borkin et al. 2002). Formerly, it was found in Serbia only at the Obedska bara locality (Spasić Bošković et al. 1999). However, in our investigations, we found this system in four localities: 1, 2, 3 and 9, all within the Danube River area (Fig. 1).

Although the L-E system represents the most frequent genome combination encountered in central Europe (see Graf & Polls-Pelaz 1989, Berger 1990, Plötner & Grunwald 1991, Rybacki & Berger 2001, Pagano et al. 2001), we recorded this system in only one locality (Fig. 1). Previously, in the same region (Spasić 1996), *P. ridibundus* individuals were detected at a frequency 20% of the water frog population, while three years later, their frequency was only 3% in the population samples that we analysed (Spasić Bošković et al. 1999). It is important to point out that we found only

*P. kl. esculentus* females at this locality. This finding indicates the possibility that the hybridogenetic females that cross with the males produce only *esculentus* females (Tunner 1974, 1980). At the same time, the L-E population system was found at only two localities close to the Danube River (Spasić Bošković et al. 1999). Longstanding uncontrolled hunting of and trade in water frogs (Džukić et al. 2001) are serious threats that could lead to the extinction of this taxa through causing changes to their population structures and to the type of population systems that are encountered. We believe that the L-E system might also be found towards the north, in the Vojvodina province. This presumption is based on the published records of similar population systems in the surrounding areas (Berger 1990, Gubányi & Korsós 1992, Tunner & Heppich Tunner 1992).

Populations belonging to the R-E system are less widespread than L-E populations (Graf & Polls-Pelaz 1989). Nevertheless, they have been found throughout a considerable part of the range of the water frog

complex (Uzzell & Berger 1975, Berger 1990, Tunner & Heppich Tunner 1992, Plötner et al. 1994, Pagano et al. 2001, Rybacki & Berger 2001, Borkin et al. 2004a, b). R-E populations occurred predominately within the distribution area of their parent species. We found the R-E system only outside of the *P. lessonae* distribution range.

Overall, the R-E population system was found to spread southwards from the Sava and the Danube Rivers, apart from locality No. 4 that was located on the left bank of the Danube River. At all of the investigated localities where the R-E population systems were recorded, *P. ridibundus* was the numerically more abundant species (Fig. 1). Abundance of the hybrid “species” was considerably lower towards the south, where it was found at a frequency of approximately 20% overall. In particular, hybrids were found southwards from the Western Morava River and the town of Zaječar. However, in locality No. 12, further to the south, the abundance of *P. kl. esculentus* increased (26%). Contrary to our expectation to find the R-E-L and L-E population systems of water frogs north of the Sava and the Danube Rivers along with the “pure” *esculentus-esculentus* populations (Spasić Bošković et al. 1999), we did not find any record of “pure” populations of any of the taxa that we studied at any locality.

The sex ratio of the hybrid taxa is an important characteristic of the R-E system (Uzzell et al. 1977, Rybacki 1994a, b, Pagano et al. 1997, Ragghianti et al. 2007). There was a declining trend in the number of females compared to males *P. kl. esculentus* from the north to the south. In northern populations the percentage of females varied from 33.3% (loc. 1.) to 100% (loc. 15.). In southern populations, these percentages varied from 20% (loc. 10.) to 0% (localities

11, 13 and 14). The source of this sex-ratio variation is unknown. Previous studies suggested that R-E systems with hybrid males may experience exclusion of both the *ridibundus* and *lessonae* genomes, such that, they will produce sperm only containing the *lessonae* or *ridibundus* genome, or, possibly, containing a mixture of both genomes (see Ragghianti et al. 2007). Investigations of the R-E system have also indicated the existence of populations with different sex and genetic structures and the possibility of exclusion of either the *ridibundus* or *lessonae* genomes in male hybrid forms (Uzzell et al. 1977, Rybacki 1994a, b, Pagano et al. 1997, Ragghianti et al. 2007).

In conclusion, our investigations suggest that areas north of the Danube and Sava Rivers are inhabited with all three taxa. However, the proportion of taxa in mixed populations and their sex ratios varied substantially within the study area. The limited presence of a hybrid “species” with extremely rare females, in the central and southern parts of the investigated area, may indicate that (1) *P. ridibundus* fluctuate geographically in the capability of its genome to induce hybridogenetic gametogenesis (Hotz et al. 1985) and (2) *lessonae* and *ridibundus* genomes were either excluded or mixed in male hybrid forms (Ragghianti et al. 2007).

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### Literature

- Beerli P. 1994: Genetic isolation and calibration of an average protein clock in western Palearctic water frogs of the Aegean region. *Inaugural Dissertation, Philosophischen Fakultät, Zurich*: 92.
- Berger L. 1990: On the origin of genetic system in European water frog hybrids. *Zoologica Poloniae* 35 (1–4): 5–31.
- Borkin L.J., Litvinchuk S.N., Mannapova E.I., Pestov M.V. & Rosanov J.M. 2002: The distribution of green frogs (*Rana esculenta* complex) in Nizhny Novgorod Province, Central European Russia. *Russian J. Herpetol.* 9 (3): 195–208.
- Borkin L.J., Litvinchuk S.N., Rosanov J.M. & Skorinov V.D. 2004a: On cryptic species (an example of Amphibians). *Entomol. Rev.* 84: S75–S98.
- Borkin L.J., Korshunov V.A., Lada A.G., Litvinchuk S.N., Rosanov J.M., Shabanov A.D. & Zinenko I.A. 2004b: Mass occurrence of polyploid green frogs (*Rana esculenta* complex) in eastern Ukraine. *Russian J. Herpetol.* 11 (3): 194–213.
- Colganiceanu D. & Tesio C. 1993: On the presence of *Rana lessonae* in Rumania. *Amphibia-Reptilia* 14: 90–93.
- Džukić G., Kalezić M.L., Ljubisavljević K.M. & Krizmanić I. 2001: The integrative analysis of exploitation of the green frogs in SR Yugoslavia. *The Final Reports I & II. Federal Secretariat of Labour, Health and Social*

*Care, Serbia: 99. (in Serbian)*

- Graf J.D. & Polls-Pelaz M. 1989: Evolutionary genetics of the *Rana esculenta* complex. In: Dawley R.M. & Bogart J.P. (eds.), Evolution and ecology of unisexual vertebrates. *New York State Mus Bull* 466, New York: 289–302.
- Gubányi A. & Korsós Z. 1992: Morphological analysis of two Hungarian water frog (*Rana lessonae-esculenta*) populations. *Amphibia – Reptilia* 13: 235–243.
- Günther R., Plötner J. & Tetzlaff I. 1991: Zu einigen Merkmalen der Wasserfrösche (*Rana* synkl. *esculenta*) des Donau – Deltas. *Salamandra* 27: 246–265.
- Hotz H., Mancino G., Bucci-Innocenti S., Raghianti M., Berger L. & Uzelli T. 1985: *Rana ridibunda* varies geographically in inducing clonal gametogenesis in interspecies hybrids. *J. Exp. Zool.* 236: 199–210.
- Karaman L.S. 1948: Prilog herpetologiji sjeverne Srbije. *Prirodoslovna istraživanja JAZU* 24: 51–73.
- Kotlík P. 1996: Hybridogenesis in water frogs: Genetic analysis of populations of *Rana esculenta* complex in the Czech Republic (Amphibia, Ranidae). *Thesis, Department of Zoology, Faculty of Science, Charles University in Prague.*
- Krizmanić I. 2008: Water frogs (*Rana esculenta* complex) in Serbia – morphological data. *Arch. Biol. Sci.* 60: 449–457.
- Lada G.A., Borkin L.J. & Vinogradov E.A. 1995: Distribution, population system and reproductive behavior of green frogs hybridogenetic (*Rana esculenta* complex) in Central chernozem territory of Russia. *Russian J. Herpetol.* 2: 146–157.
- Lode T. & Pagano A. 2000: Variations in call and morphology in male water frogs: Taxonomic and evolutionary implications. *C. R. Acad. Sci., Ser. III* 323: 995–1001.
- Miller M.P. 1997: Tools for population genetic analysis (TFPGA) 13. *Department of Biological Sciences, Northern Arizona University, Flagstaff.*
- Murphy R.W., Sites J.W., Buth D.G. & Haufler C.H. 1996: Proteins – isozyme electrophoresis. In: Hillis et al. (eds.), Molecular systematics. *Sunderland, Sinauer Associates Inc, 2nd ed. Massachusetts: 51–120.*
- Pagano A. & Joly P. 1999: Limits of the morphometric method for field indentification of water frogs. *Alytes* 16 (3–4): 130–138.
- Pagano A., Crochet P.A., Graf J.D., Joly P. & Lode T. 2001: Distribution and habitat use of water frog hybrid complexes in France. *Global Ecology & Biogeography* 10: 433–441.
- Pagano A., Joly P. & Hotz H. 1997: Taxon composition and genetic variation of water frogs in the Mid-Rhone floodplain. *C. R. Acad. Sci., Ser. III* 320: 759–766.
- Plötner J. & Grunwald C. 1991: A mathematical model of the structure and the dynamics of *Rana ridibunda/esculenta* -♂♂- populations (Anura, Ranidae). *Z. Zool. Syst. Evolut.-forsch.* 29: 201–207.
- Plötner J., Becker C. & Plötner K. 1994: Morphometric and DNA investigations into European water frogs *Rana* kl. *esculenta* synklepton (Anura, Ranidae) from different population systems. *J. Zool. Syst. Evol. Res.* 32: 193–210.
- Radovanović M. 1951: Vodozemci i gmizavci naše zemlje [Amphibians and reptails our country]. *Naučna knjiga, Beograd. (in Serbian)*
- Radovanović M. 1964: Die Verbreitung der Amphibien und Reptilien in Jugoslawien. *Senck. Biol.* 45 (3/5): 553–561.
- Raghianti M., Bucci S., Marracci S., Casola C., Mancino G., Hotz H., Guex D.G., Plötner J. & Uzelli T. 2007: Gametogenesis of intergroup hybrids of hemiclinal frogs. *Genet. Res.* 89: 39–45.
- Rybacki M. (1994a): Structure of water frogs populations (*Rana esculenta* complex) of the Wolin Island, Poland. *Zoologica Poloniae* 39 (3–4): 345–364.
- Rybacki M. (1994b): Water frogs (*Rana esculenta* kompleks) of the Bornholm Island, Denmark. *Zoologica Poloniae* 39 (3–4): 331–344.
- Rybacki M. & Berger L. 2001: Types of frog populations (*Rana esculenta* complex) in Poland. *Mitt. Mus. Natkd. Berl. Zool. Reiche* 771: 51–57.
- Schneider H., Sofianidou T.S. & Kyriakopoulou-Sklavounou P. 1984: Bioacoustic and morphometric studies in water frogs (genus *Rana*) of Lake Ioannina in Grece, and description of a new species (Anura, Amphibia). *Z. Zool. Syst. Evolut.-forsch.* 22: 349–366.
- Spasić O. 1996: Komparativna citogenetička analiza hromozomskih komplementa vrsta roda *Rana* (Anura, Amphibia) sa područja Jugoslavije [Comparative cytogenetic analysis of species of the genus *Rana* (Anura, Amphibia) sa područja Jugoslavije]

- Amphibia) from Yugoslavia]. *Magister thesis, Faculty of Biology, University of Belgrade. (in Serbian)*
- Spasić Bošković O., Krizmanić I. & Vujošević M. 1999: Population composition and genetic variation of water frogs (Anura: Ranidae) from Yugoslavia. *Caryologia* 52: 9–20.
- StatSoft INC. 2001: Statistica for Windows 6.0 (Computer program manual). *Tulsa 3rd ed.*
- Tunner H.G. 1974: Die Klonale struktur einer Wasserfrösche population. *Z. Zool. Syst. Evolut.-forsch.* 10: 127–132.
- Tunner H.G. 1980: Kreuzungsexperimente mit Wasserfröschen aus österreichischen und polnischen Mischpopulationen (*Rana lessonae* + *Rana esculenta*) – Eine Analyse biochemischer und morphologischer Merkmale. *Z. Zool. Syst. Evol.* 18: 257–297.
- Tunner H.G. & Heppich Tunner S. 1992: A new population system of water frogs discovered in Hungary. In: Korsós Z. & Kiss I. (eds.), *Proceedings of the Sixth Ordinary General Meeting of the Societas Europaea Herpetologica, Budapest: 453–460.*
- Uzzell T. & Berger L. 1975: Electrophoretic phenotypes of *Rana ridibunda*, *Rana lessonae* and their hybridogenetic associate *Rana esculenta*. *Proc. Acad. Nat. Sci. Philadelphia* 127 (2): 13–24.
- Uzzell T., Günther R. & Berger L. 1977: *Rana ridibunda* and *Rana esculenta*: A leaky hybridogenetic system (Amphibia, Salientia). *Proc. Acad. Nat. Sci. Philadelphia* 128: 147–171.