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AMPHIBIANS IN SERBIA – DISTRIBUTION AND DIVERSITY PATTERNS

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Considering the need for an up-to-date overview of the distribution and diversity of amphibians of Serbia, we mapped the species distributions upon current, mostly unpublished, faunistic data. Also, we compared batrachofaunas of Serbia and other Balkan countries in terms of species number and zoogeographic batrachofaunistic elements. With its 21 native species (8 urodeles and 13 anurans), Serbia appears to be the second most diverse country in the Balkans. We found three main centres of species richness in Panonnian and Peripannonian Serbia (with 15-17 species per 50 x 50 *km* square) which signals urgency for their protection. Serbian batrachofauna is most similar to that of the eastern Balkan countries (Bulgaria and Romania). Zoogeographic analysis showed that amphibian fauna of Serbia consists of six chorotypes, with the central-European and southern--European as the most dominant ones.

Key words: amphibian diversity, distribution, zoogeography

INTRODUCTION

Data about the distribution of species is basic for use in future research in the areas of ecology, systematics, biogeography and conservation biology. Regarding amphibians, their rapid worldwide declines have reinforced a need for more precise data about their distribution at the regional and national levels, especially in countries with poor distributional data (e.g. Margules *et al.* 2002). Additionally, understanding of the spatial variability in species richness is very valuable for conservation planning (e.g. Schouten *et al.* 2009). Nowadays, it is widely recognized that the identification and conservation of specific important areas, especially those featuring exceptional concentrations of species (hotspots), is of prime importance in efforts to reduce the loss of any biological diversity (e.g. Buse & Griebeler 2012). Therefore, an important challenge in conservation biology is to identify those areas on both large and small geographic scales.

The entire territory of Serbia, taking into account both the geographical location as well as the elevation gradient, is inhabited by amphibians. Nonetheless, to date no general account (even on a large spatial scale; but see Kalezić & Džukić 2001) of distribution patterns, species diversity and zoogeographic analyses of all Serbian amphibians has been undertaken. This is in sharp contrast to the "value" of Serbian batrachofauna in regards to a high level of species richness comparing to other Balkan s countries (see below), as well as to a number of important biological peculiarities.

Concerning batrachofauna, Serbia is an area with (1) ancient phylogeographic clades, (2) centre of species origin, (3) speciation and (4) microrefugia presence (e.g. alpine newts, Sotiropoulos *et al.* 2007; crested newts, Arntzen *et al.* 2007; fire-bellied toads, Hofman *et al.* 2007). In the region of Serbia, there are numerous examples of peripheral amphibian habitat segments (see below); for this reason this region is of particular importance in the protection and preservation of peripheral populations which are extremely sensitive to changes within the ecosystems they inhabit (e.g. Kawecki 2008). Serbia harbours those rare regions in which the narrow zone of sympatry of some amphibian groups (e.g. zones of the habitats of spadefoot toads, Džukić *et al.* 2005) and, in some instances, of syntopy as well occurs. Also, it has been proposed that the emergence and speciation of some amphibian groups take place, at least in part, in Serbia (e.g. crested newts, Ivanović *et al.* 2012). These amphibian groups are important as model-organisms in various biological research areas (e.g. evolutionary biology, population and conservation biology). Of special significance in terms of conservation are relict populations in some parts of Serbia, such as the Syrian and common spadefoot toad (Džukić *et al.* 2005). A prominent characteristic of Serbian batrachofauna is also the presence of vicariance

(the existence of phylogenetically close taxa in different geographical areas as a result of the formation of a natural biogeographical barrier within a previously integrated area) in eastern Serbia where *Rana graeca* and *R. temporaria* vicariate (Džukić, unpubl.). The evolutionary phenomenon of facultative paedomorphosis (absence or significant reduction of metamorphosis during life cycle), which are of especial interest from the point of view of conservation (see Denoël *et al.* 2009), has also been established in a number of smooth newt (*Lissotriton vulgaris*) populations in the Pannonian Plain (Džukić *et al.* in preparation). Yet in spite of all these facts, Serbia still lacks a comprehensive review of amphibian species.

The aims of this study were to: (1) present an updated and annotated checklist of amphibian species in Serbia; (2) provide general distribution maps of all species, including the characteristics of distribution patterns in terms of continuity and marginality; (3) analyse species richness at different spatial scales and along elevation gradients; (4) provide zoogeographic analysis of Serbian amphibians.

MATERIAL AND METHODS

Identification of the majority of species was done according to the standard herpetological literature (e.g. Arnold & Ovenden, 2002). Due to difficulties in taxonomic reorganization, species identification of some groups was mainly based on spatial distribution of presumably diagnostic morphological traits, diagnostic allozyme characters for green frogs (Krizmanić 2008; Krizmanić & Ivanović 2010) and morphological and genetic analyses for *Triturus cristatus* s.l. (e.g. Kalezić *et al.* 1997, Wielstra *et al.* 2013a).

Distribution of Serbian amphibian species is based on georeferenced species occurrences which include our approximately 5000 items of distributional data which have been collected from different sources. About 4200 of these items came from (1) the Batrachological Collection of the Institute for Biological Research "Siniša Stanković" (Belgrade), (2) our own unpublished field data, as well as (3) unpublished data which were kindly donated by our colleagues (see Acknowledgements). In addition, we used already published data (around 800 references; pdf files available upon request). These distributional data we believe represent fairly well not only the species presence, but also the characteristics of species ranges in Serbia. Most reported outliers which have not been confirmed by us were not considered in further analyses. We presented two types of species' distribution patterns: 1) inferred distribution area (bright green in Figs. 1-7) - based on our knowledge of the suitable aquatic and terrestrial habitats where the species occurrence has not been confirmed yet, 2) confirmed distribution area (dark green in Figs. 1-7) - based on actual species occurrences.

As we still lack faunistical data from some Serbian regions (see Discussion), and for the purpose of this article, amphibian distributions in Serbia were shown as global maps of species ranges rather than as maps with point locality data, an approach which garners support from the recent claim that global amphibian assessment range maps represent their distribution fairly well (Ficetola *et al.* 2013).

In order to reduce potential bias in sampling effort as well as to visualize regional patterns better (see Graham & Hijmans 2006), species richness was assessed at three coarser levels: (1) at 50 × 50 *km* squares of the UTM National Greed Reference, (2) according to biogeographic regions (Marković, 1970; Stevanović 1992, see below), and (3) altitudinal and latitudinal divisions of Serbia – Pannonian, Peripannonian, Mountain--valley Serbia (Marković 1970). For analyses of amphibian diversity and designation of the centres of the batrachological diversity in Serbia, we used an application created in Visual Basic 6.1 in the program WinWord 2003 (Niketić 1999), using the method by Walter & Straka (1970) at National Greed UTM Reference for Serbia 50 x 50 *km*. Similarities among the regions of Serbia and with the Balkan countries were taken according to the Bray-Curtis similarity index (Ludwig & Reynolds 1988). Data on the presence of the taxa for Bosnia and Herzegovina, Greece, Bulgaria, Montenegro and Romania are taken from Gasc *et al.* (1997), Valakos *et al.* (2008), Jablonski *et al.* (2012), Stojanov *et al.* (2011), Polović & Ljubisavljević (2010), and Cogălniceanu *et al.* (2013).

The biogeographic regions (Marković, 1970, Stevanović, 1992) of Serbia are: Bačka (Ba), Banat (Bt), Srem (Sr), Pomoravlje (Po), Šumadija (Š), central Serbia (C), north-eastern Serbia (NE), eastern Serbia (E), north--western Serbia (NW), south-eastern Serbia (SE), western Serbia (W), south-western Serbia (SW), southern Serbia (S), Kosovo (K), and Metohija (M) (see Fig. 9).

As mentioned above, there are three altitudinal Serbian regions which are distinctive in their geographical and ecological aspects - (1) the Pannonian region (low-lying region north of the Sava and Danube Rivers, up to 200 *m* above sea level), (2) the Peripannonia region (the low-lying region and hills south of the Sava and Danube, from 200 to 600 *m* above sea level), and (3) the Mountain-valley region (the central and southern parts of Serbia, from 600 up to 2650 *m*) (see Fig. 9). Pannonian and Peripannonian areas are about the same surface area (22.200 km^2 and 23.300 km^2, respectively), while the mountains region is much larger (42.800 km^2).

For zoogeographic analyses, chorotypes were identified according to the classification of Vigna Taglianti *et al.* (1999). Regarding the taxonomic issues, we followed suggestions of Speybroeck *et al.* (2010).

RESULTS

Species richness and distribution

The list of amphibians in Serbia includes 21 native species: eight species of Caudata and 13 species of Anura (Tab. 1). This species diversity puts Serbia in second place among the Balkan countries, closely following Greece which has 22 native amphibian species (Valakos *et al.* 2008).

Table 1. - List of Amphibian species in Serbia.

Order	Family	Species	Marginal zone
Caudata	Salamandridae	*Salamandra atra*	+
		Salamandra salamandra	+
		Ichthyosaura alpestris	
		Lissotriton vulgaris	+
		Triturus cristatus	+
		Triturus dobrogicus	+
		Triturus karelinii	+
		Triturus macedonicus	+
Anura	Hylidae	*Hyla arborea*	
	Bombinatoridae	*Bombina bombina*	+
		Bombina variegata	+
	Bufonidae	*Bufo bufo*	
		Pseudepidalea viridis	
	Ranidae	*Pelophylax lessonae*	+
		Pelophylax kl. *esculentus*	+
		Pelophylax ridibundus	
		Rana graeca	+
		Rana dalmatina	
		Rana temporaria	+
	Pelobatidae	*Pelobates fuscus*	+
		Pelobates syriacus	+

The most widely distributed species that occupy the entire territory of Serbia are urodela *L. vulgaris*, and five anuran species (*Bufo bufo, Pseu-*

depidalea viridis, Hyla arborea, Rana dalmatina and *Pelophylax ridibundus*). Species that inhabit more than 50% of Serbia are three tailed amphibians (*Salamandra salamandra, Ichthyosaura alpestris* and *Triturus macedonicus*), and two anurans (*Bombina variegata* and *Pelophylax* kl. *esculentus*). Rare amphibian species, which occupy 10 – 50% of the country, are: *Triturus dobrogicus* and *T. karelinii* among tailed amphibians, and five species of anurans (*Bombina bombina, Pelobates fuscus, Pelophylax lessonae, Rana graeca* and *R. temporaria*). Species with extremely limited distribution in Serbia (less than 10% of the territory) are: *Salamandra atra*, *Pelobates syriacus* and *Triturus cristatus* (Figs. 1-7).

Among amphibians which are not widespread in Serbia, eight species show fragmented ranges (*Ichthyosaura alpestris*, *Triturus cristatus*, *T. karelinii*, *Salamandra salamandra*, *Bombina variegata*, *Rana temporaria*, *Pelobates fuscus* and *P. syriacus*). The most common pattern of fragmentation of distributional ranges is one larger fragment and one or two small disjunctions (see Figs. 1-7). The most prominent deviation from this pattern is *Rana temporaria* with a more fragmented range than in other amphibian species. Notably, this species shows much more phylogeographic substructuring in comparison with *R. dalmatina* whose overall distribution is homogenous not only in Serbia, but across Europe as well (Vences *et al.* 2013).

A noteworthy feature of amphibian ranges is that 15 species of a total 21 species (= 71%) are within the marginal zone of their distributional range (see Tab. 1). Edge populations may be of greater conservation priority because of their potentially unique genetic characteristics and/or because they are highly vulnerable to loss of genetic diversity (Vucetich & Waite 2003); as well, rear edge populations might be of special importance for evolutionary processes, not only on the species level but for the evolution of biota as well (Hampe & Petit 2005).

Species hot-spots

Analysis of amphibian biodiversity in Serbia at 50 × 50 *km* UTM National Greed Reference showed that the highest number of species (15--17 species) was found in three 50 × 50 *km* UTM squares: (1) EP1 – in the Velika Morava region; (2) DQ2 – north-western Serbia; (3) EQ1 – south--eastern Banat. In contrast, the lowest species diversity appeared in 12 UTM squares with less than 10 species per 50 x 50 *km*, predominantly in the northern parts of the country, as well as in the bordering areas (Fig. 8).

Analysis of amphibian biodiversity in Serbia at an altitudinal geographic division showed that Peripannonian and Mountain-valley parts of Serbia are characterized by a higher number of amphibian species (19) (Fig. 9).

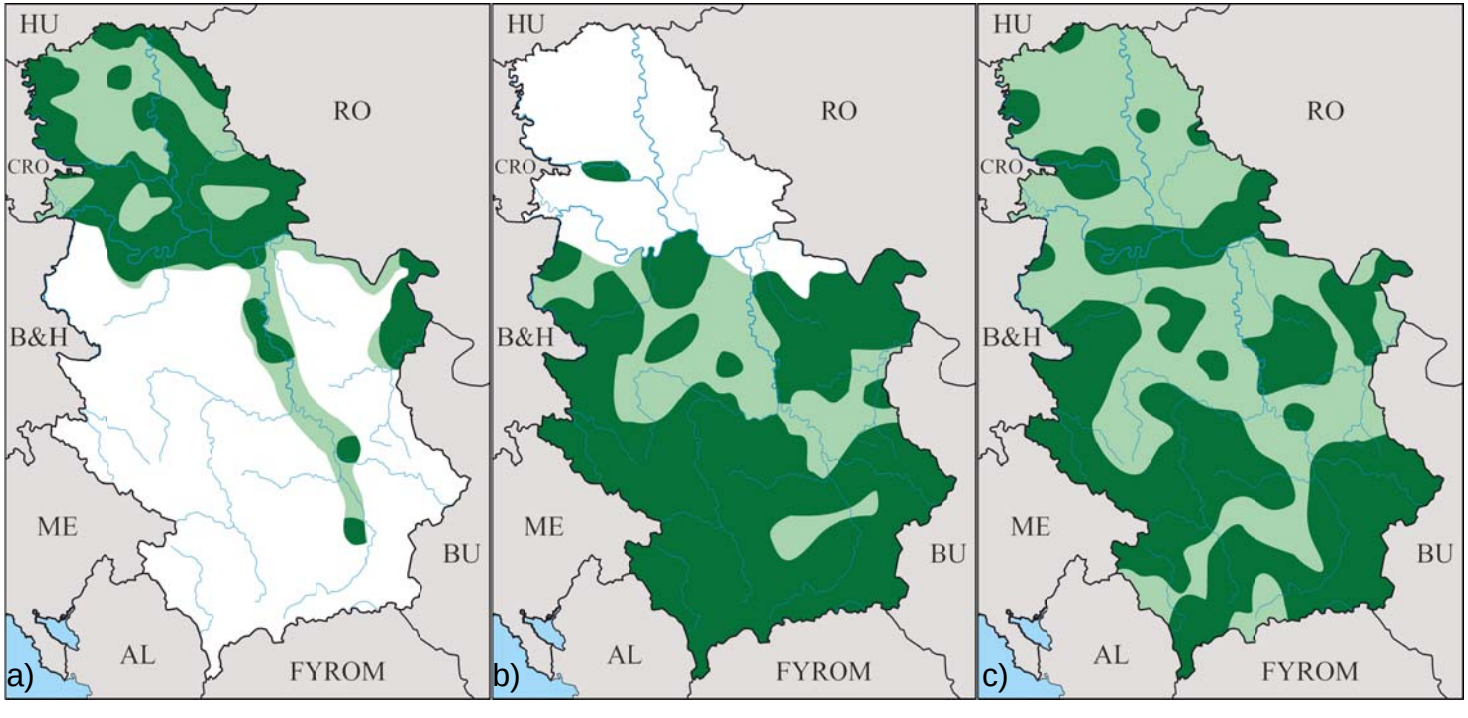

Fig.1. - Distribution range of :a) *Bombina bombina*, b) *Bombina variegata*, c) *Bufo bufo* in Serbia.
(bright green: inferred distribution area, dark green: confirmed distribution area)

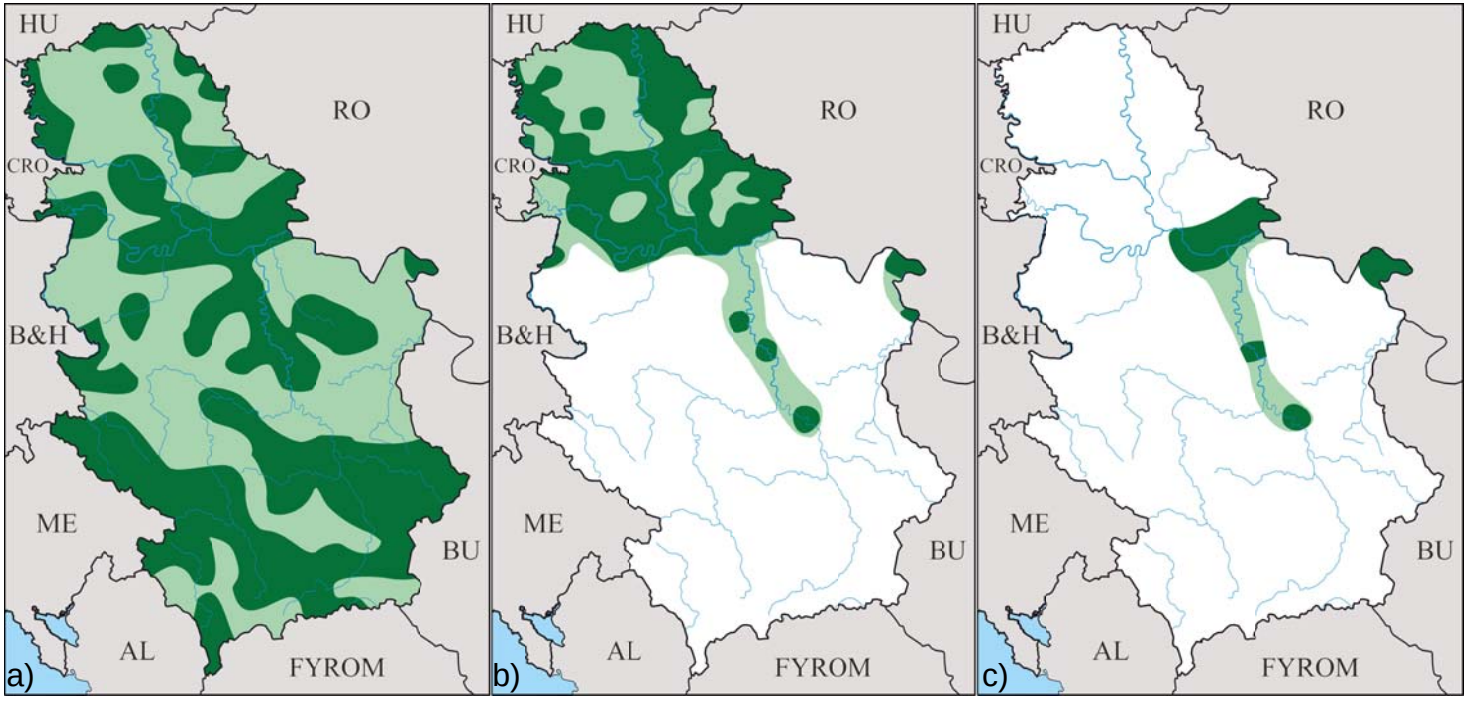

Fig.2. - Distribution range of: a) *Pseudepidalea viridis*, b) *Pelobates fuscus*, c) *Pelobates syriacus* in Serbia. (bright green: inferred distribution area, dark green: confirmed distribution area)

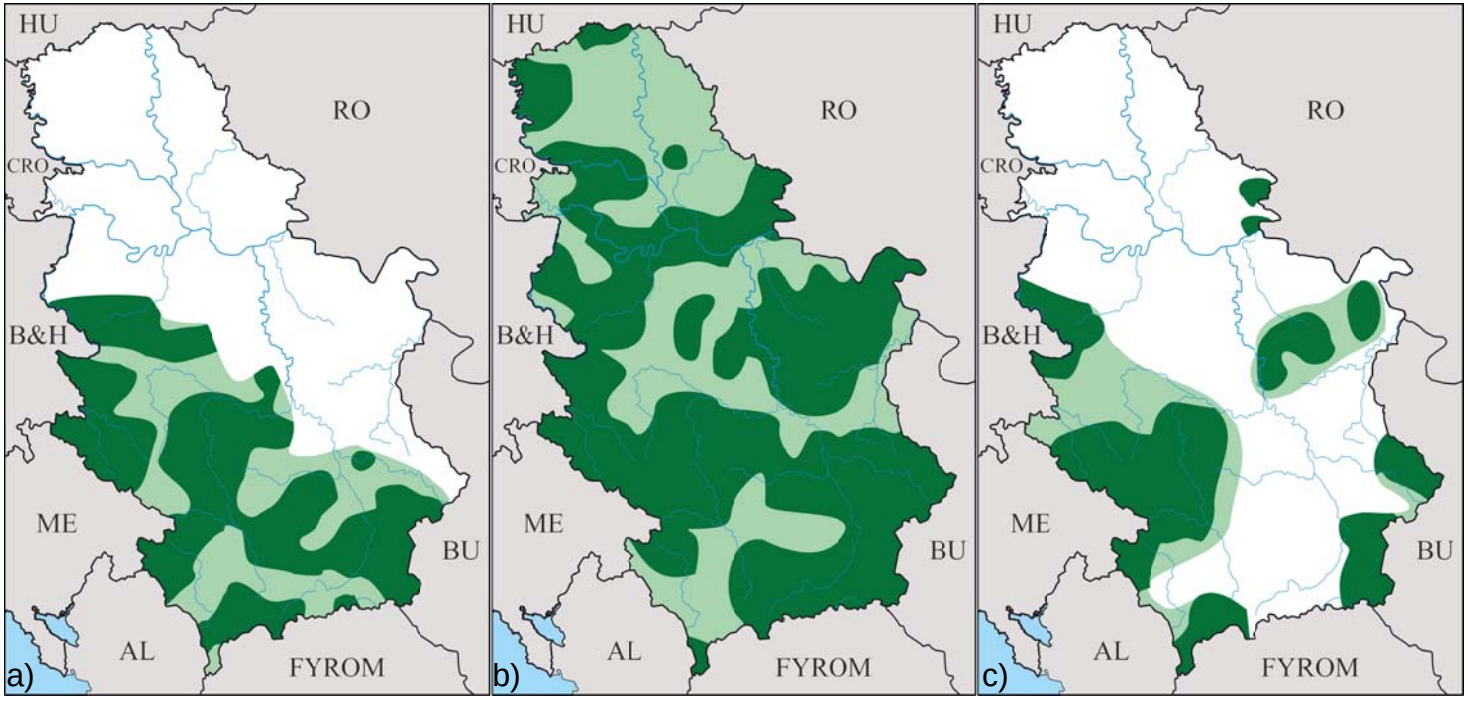

Fig. 3. - Distribution range of: a) *Rana graeca*, b) *Rana dalmatina*, c) *Rana temporaria* in Serbia. (bright green: inferred distribution area, dark green: confirmed distribution area)

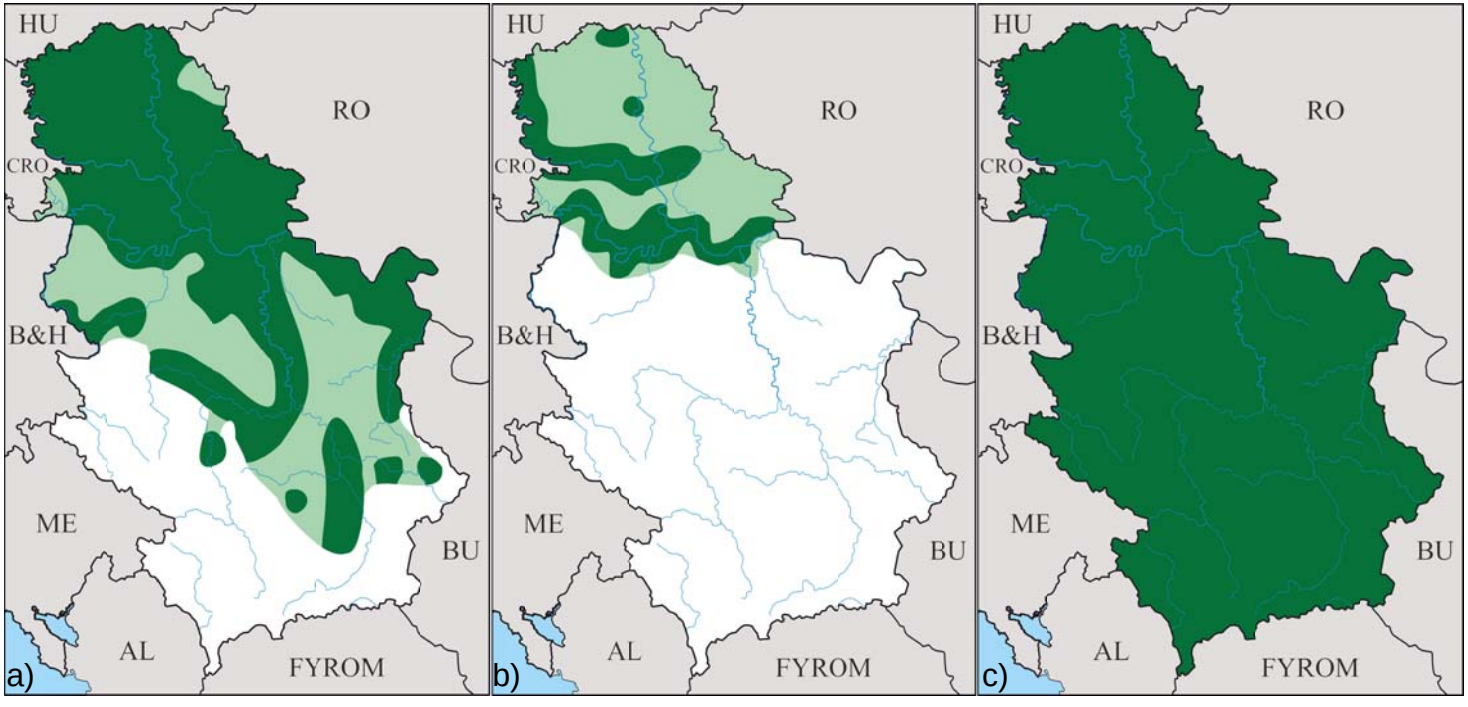

Fig 4. - Distribution range of: a) *Pelophylax* kl. *esculentus*, b) *Pelophylax lessonae*, c) *Pelophylax ridibundus* in Serbia. (bright green: inferred distribution area, dark green: confirmed distribution area)

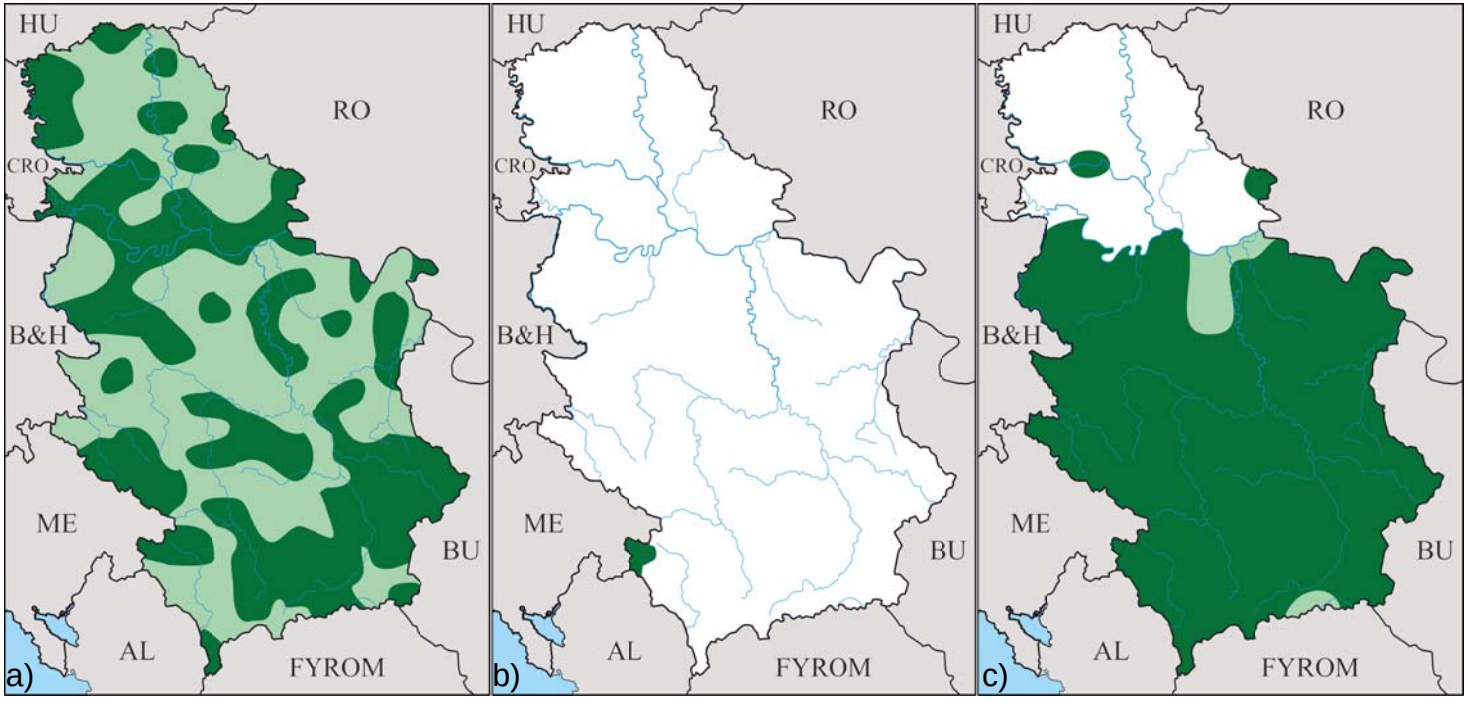

Fig. 5. - Distribution range of: a) *Hyla arborea*, b) *Salamandra atra*, c) *Salamandra salamandra* in Serbia. (bright green: inferred distribution area, dark green: confirmed distribution area)

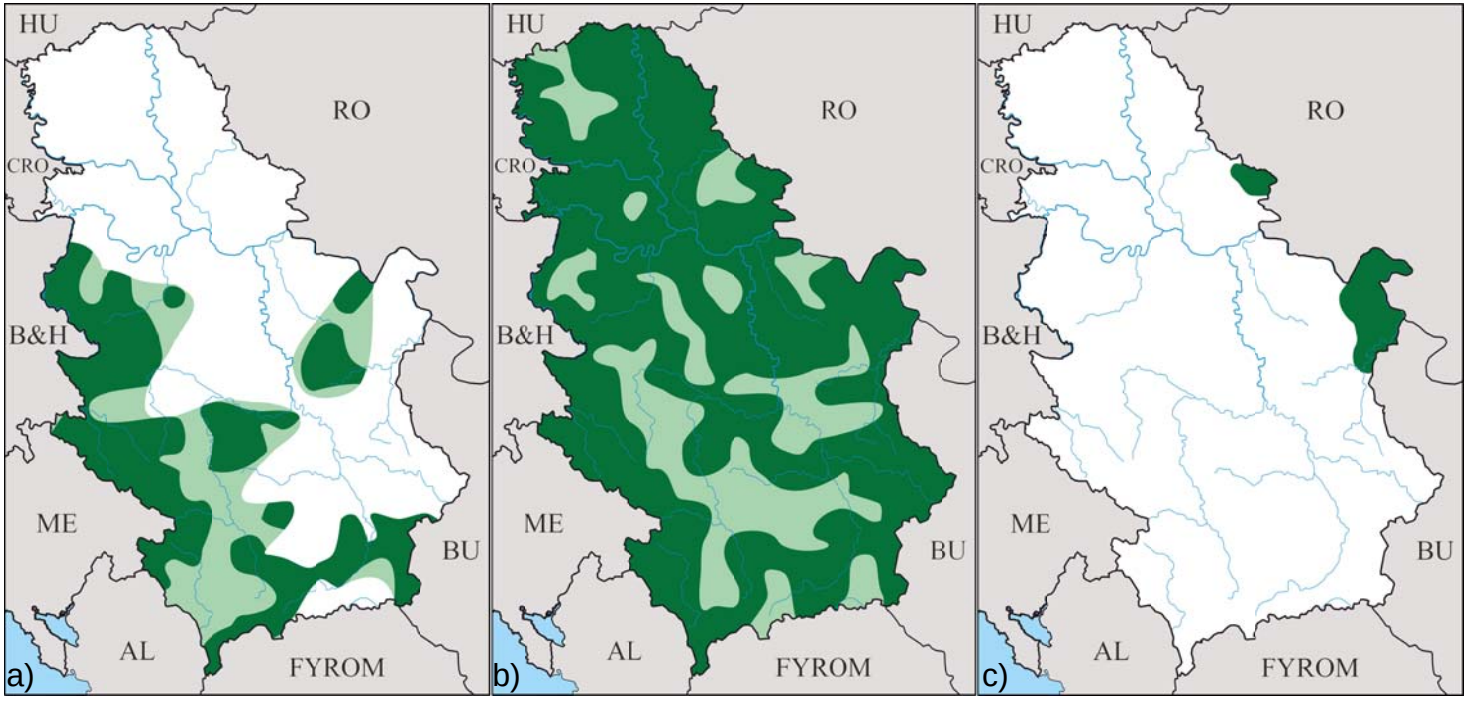

Fig. 6. - Distribution range of: a) *Ichthyosaura alpestris*, b) *Lissotriton vulgaris*, c) *Triturus cristatus* in Serbia. (bright green: inferred distribution area, dark green: confirmed distribution area)

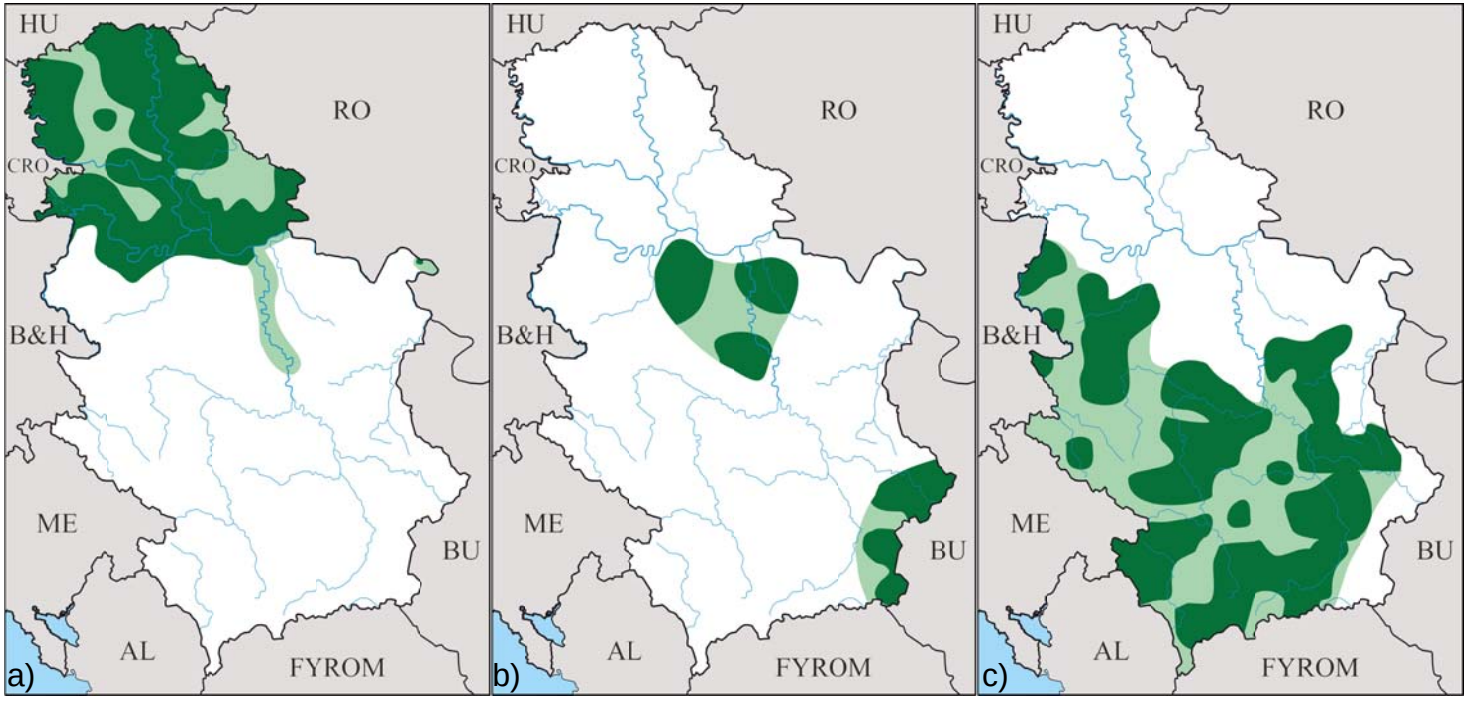

Fig. 7. - Distribution range of: a) *Triturus dobrogicus*, b) *Triturus karelinii*, c) *Triturus macedonicus* in Serbia. (bright green: inferred distribution area, dark green: confirmed distribution area)

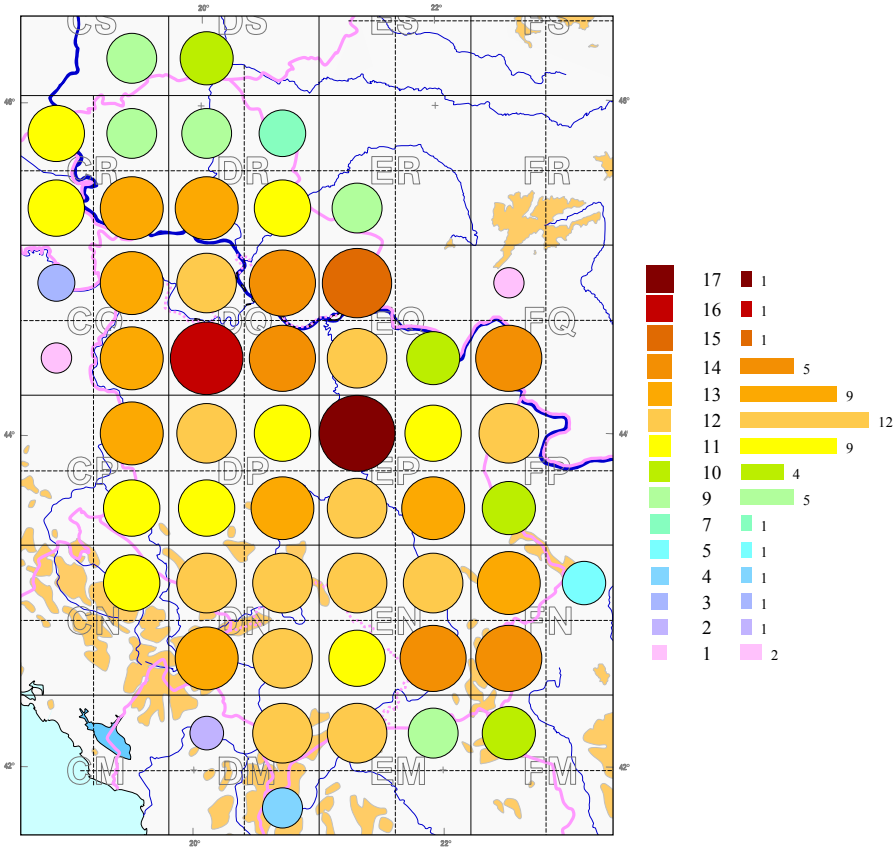

Fig. 8. - Species richness of Amphibians in Serbia at National Grid UTM 50 × 50 *km* Reference.

According to the analysis of amphibian diversity at the regional level of Serbia, north-western and eastern parts, as well as Šumadija have the highest number of species (17). Bačka is the region with the lowest number of amphibian species (11) (Fig. 9), which might be an artefact of insufficient sampling (see below).

Faunal similarities

The Bray-Curtis similarity index showed that parts of Pannonian (Bačka, Srem and Banat) and Peripannonian Serbia (north-western Serbia, Šumadija and Pomoravlje) are the most similar to each other, while other regions of mountain-valley Serbia are most distinct in terms of amphibian fauna (Fig. 10).

The same similarity index was also used for comparative analysis of amphibian fauna of Serbia with those from all the Balkan countries. Results

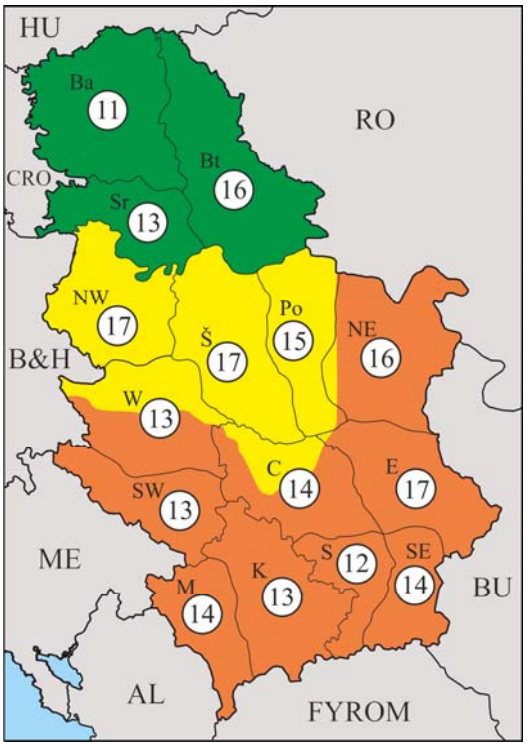

Fig. 9. - Species diversity of Amphibians in Serbia at regional level according to biogeographic (see Material and methods section for abbreviations) and altitudinal and latitudinal division (green: Pannonian, yellow: Peripannonian, orange: Mountain-valley part) of Serbia.

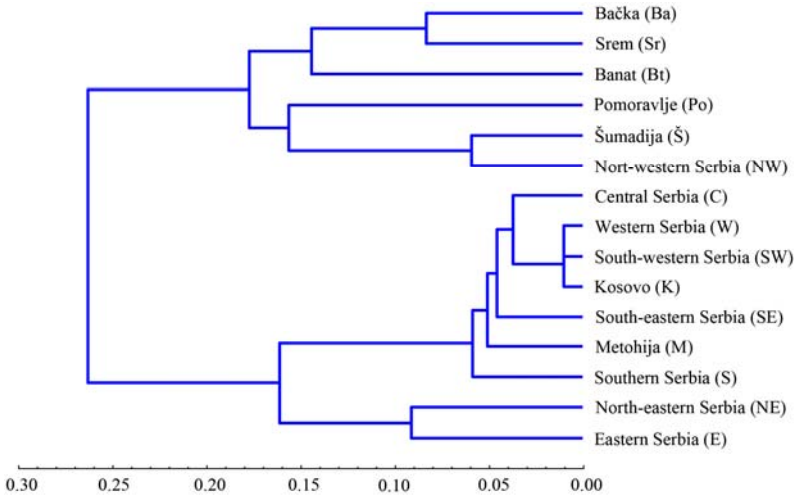

Fig.10. - Cluster diagram of Bray Curtis similarity Index of biogeographic regions in Serbia.

showed that Serbian batrachofauna is most similar to the eastern Balkan countries (first with Bulgaria, and then with Romania). This country cluster adjoins the cluster of western Balkan countries (Croatia, Bosnia and Herzegovina, Slovenia). Apparently, Serbian batrachofauna is the most unrelated to the amphibian faunas of the southern Balkans (Greek, Albanian, FYR of Macedonia and Montenegrin) (Fig. 11).

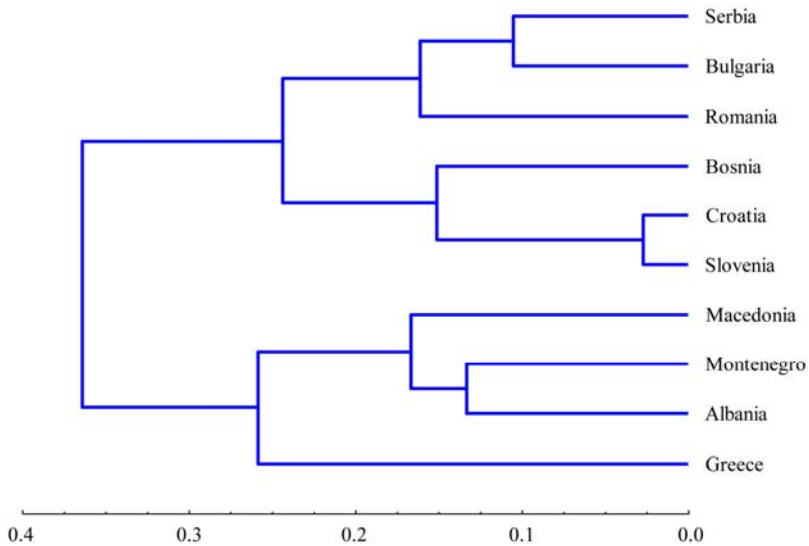

Fig.11. - Cluster diagram of Bray Curtis similarity Index of the Balkan countries.

Zoogeographic analysis showed that amphibians in Serbia were classified into six chorotypes (Tab. 2). The most dominant chorotypes were the central-European and the southern-European with six species each, followed by the Turano-European with four species and the European with three species.

DISCUSSION

Serbia is a region of transitions where faunal elements of various origins meet. Not surprisingly, amphibian communities of Serbia are noticeably rich, leading to the international recognition of the area as important for the conservation of batrachofauna (e.g. Kalezić & Džukić 2001). Of the native 33 amphibian species living on the Balkan Peninsula, one of the 25 most important species-rich areas of biodiversity (Myers *et al.* 2000), Serbia is in second place among contemporary countries in the Balkans in terms of amphibian species diversity (one species less than Gre-

Table 2 - Classification of Amphibians in Serbia according to chorotypes.

Chorotype	**No. of species**	**Species**
Europeo-Mediterranean	1	*Bufo bufo*
Turano-Mediterranean	1	*Pelobates syriacus*
European	3	*Ichthyosaura alpestris*
		Lissotriton vulgaris
		Rana temporaria
Turano-European	4	*Triturus karelinii*
		Hyla arborea
		Pseudepidalea viridis
		Pelophylax ridibundus
Central-European	6	*Triturus cristatus*
		Triturus dobrogicus
		Bombina bombina
		Pelophylax lessonae
		Pelophylax kl. *esculentus*
		Pelobates fuscus
Southern-European	6	*Salamandra atra*
		Salamandra salamandra
		Triturus macedonicus
		Bombina variegata
		Rana graeca
		Rana dalmatina

ece). Additionally, there are realistic possibilities of the existence of new morphologically amphibian cryptic taxa in Serbia.

Environmental heterogeneity, in particular habitat heterogeneity (elevation gradient, landscape heterogeneity), is very important for the spatial distribution of amphibians (e.g. Maes *et al.* 2005, Schouten *et al.* 2009, Buse & Griebeler 2012). Apparently, a high diversity of amphibian species in Serbia is a result of the versatility of geographic peculiarities (heterogeneous landscapes), in particular of climate and habitat heterogeneity (e.g. Radovanović & Mijović 2005), as well as multiple zoo-geographical links, both past and present (see Džukić *et al.* 2001, Džukić & Kalezić 2004).

The northernmost part of Serbia, the Pannonian region, is widely open to central Europe with which it shares many batrachofaunal elements like *Bombina bombina*, *Pelobates fuscus*, *Pelophylax* kl. *esculentus* and *P. lessonae*. These amphibians, including *Triturus dobrogicus*, have similar ecological demands and thus essentially similar ranges largely limited to flood plains. Furthermore, they might form a flocks of species (i.e. faunal types, see Varga 1995) which is characteristic of the batrachian community of central Europe, in particular of the Pannonian plain. Moreover, the Pannonian region (most likely the Sava River drainage) was proven to be a refugium for at least two amphibians – *Pelobates fuscus* (Litvinchuk *et al.* 2013) and the *Triturus dobrogicus* (Vörös & Arntzen 2010).

The Peripannonian region is a pronounced zone of contact between previously differentiated amphibian taxa in the southern refugia (which originated from the mountainous region), and the batrachofaunal elements of the Pannonian region (resulting in frequent hybridization – e.g. the yellow-bellied *Bombina variegata*, Vukov *et al.* 2006). In particular for Serbia, it is characteristic that the suture zone of some of the groups lies along the Velika Morava and Južna Morava Rivers valleys, deep into the south. Apart from hybridization, the suture zone is characterized by parapatry (the existence of a narrow zone of area overlap with frequent gene introgressions, for example, crested newts of the *Triturus cristatus* complex (Arntzen 2003) in which species identification is, as mentioned above, a difficult task, even if genetic data are available. Moreover, the taxonomy of the crested newts in the Balkans, particularly those of the *T. karelinii* group, is still in a state of flux. Recently, within this group a new species, *T. ivanbureschi,* has been erected (Wielstra *et al.* 2013b). The range of this species includes the most southeastern part of Serbia. However, here we adhere to a more conservative approach, considering crested newts in the southeastern part of Serbia as the *T. karelinii* species *sensu lato*.

Not all Serbian areas have sufficiently fine-scaled species distribution data. Although some areas are thoroughly searched faunistically (e.g. southeastern Serbia, especially the Vlasina region, as well as southeastern Banat), some regions remain insufficiently explored in terms of amphibian presences and distribution (e.g. most of Bačka, northern Banat, southwestern Serbia, especially the Pešter region, etc., see Figs. 1-7). Also, at least in terms of species presence, there are still possibilities of finding new species whose presence has not yet been confirmed (the moor frog, *Rana arvalis*). In addition, like *Bombina bombina,* it might be that ranges of three low-levelled species (*Triturus dobrogicus*, *Pelobates fuscus* and *P. syriacus*) might be extended much more to the south along the Velika Morava and South Morava Rivers, (see Borkin *et al.* 2005). Apparently, there is still a need for detailed amphibian inventories, as species' absence

in map grids may not be because of their absence in particular areas but may represent places in which inventories have not been conducted or in which species have not been detected yet.

Even given all these facts and information, prioritisation and subsequent designation of areas in Serbia for conservation of batrachofauna emerges as essential. We found three main centres of species richness for amphibians in Panonnian and Peripannonian parts of Serbia (Figure 8), which indicates urgency in the protection of its areas. Hotspots of Serbian batrachofauna contain 85% of the total number of species (18 of 21, Figure 8). Notably, hotspots in Serbia are the result of the overlap of the ranges of widely distributed species, rather than of having small range endemics of their own.

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ВОДОЗЕМЦИ СРБИЈЕ - РАСПРОСТРАЊЕЊЕ И ДИВЕРЗИТЕТ

ТАЊА ВУКОВ, МИЛОШ Л. КАЛЕЗИЋ, ЉИЉАНА ТОМОВИЋ, ИМРЕ КРИЗМАНИЋ, ДАНКО ЈОВИЋ, НЕНАД ЛАБУС, ГЕОРГ ЏУКИЋ

Р Е З И М Е

С обзиром на потребу за најновијим прегледом дистрибуције и диверзитета водоземаца Србије, у овом раду приказане су дистрибуције врста добијене на основу постојећих и великог броја до сада необјављених фаунистичких података. Додатно, поређена је батрахофауну Србије и осталих балканских земаља у односу на број врста и зоогеографских батрахофаунистичких елемената. Са 21 аутохтоном врстом (8 врста репатих водоземаца и 13 врста безрепих водоземаца), Србија је друга земља Балкана по диверзитету водоземаца. Утврђено је постојање три главна центра диверзитета у Панонској и Перипанонској Србији (са 15-17 врста у оквиру 50 × 50 км^2) што указује на хитност увођења мера заштите ових центара. Батрахофауна Србије је најсличнија источним балканским земљама (Бугарска и Румунија). Зоогеографска анализа показала је да се фауна водоземаца Србије састоји од шест хоротипова, са централноевропским и јужноевропским као доминантним хоротиповима.