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Conservation Issues of Serbian Amphibians Identified from Distributional, Life History and Ecological Data

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Abstract: Based on 15 parameters related to the distribution, ecology and life history traits in all 21 Serbian amphibian species, we considered some relevant conservation issues. Due to the exceptionally small range of *Salamandra atra* in Serbia and the high vulnerability of this species to external threats, we considered it to be in particular danger. Another species, *Triturus dobrogicus*, which occurs in uniform lowland habitat where intense agriculture and amelioration are readily practiced, has also been rated as vulnerable in Serbia. Other crested newts (*Triturus cristatus*, *T. macedonicus* and *T. ivanbureschi*), because of the intensive loss of aquatic habitats and deterioration of the terrestrial habitats, are regarded near threatened. The three remaining species of caudate amphibians (*Salamandra salamandra, Ichthyosaurus alpestris,* and *Lissotriton vulgaris*) do not appear to be in special danger. Among the anurans, four species (*Pelobates fuscus, Pelobates syriacus, Pelophylax lessonae* and *Rana graeca*), for a variety of reasons, are under a permanent danger. It seems that the other anurans do not face immediate threat.

Keywords: Anurans, distribution, ecology, species at risk, Serbia, urodeles

Introduction

The crisis with the global amphibian declining emphasised the importance of evaluating the species conservation status not only at a larger scale, but also at the national level (*e.g.* Temple *et al.* 2009). This is particularly valid for the countries with high species richness, as well as where many particularities in amphibian biology have been found.

So far, the conservation status of Serbian amphibians has not been considered (see Kalezić, Džukić 2001, for a review on this issue), although this status is of special interest due to several facts. Of the 30 amphibian species living on the Balkan Peninsula, the batrachofauna of Serbia comprises 21 species (70%), which allows to consider it as a country with high species diversity among the European countries (Džukić, Kalezić 2004). Further grounds for regarding Serbia as a country of particular interest in terms of amphibian conservation are as follow: (1) the pronounced genetic and morphological diversity in amphibians, which resulted from the existence of numerous refuge habitats at times of climate change, especially during Pleistocene, when, in allopatric conditions, microevolutionary processes caused significant differentiation within many amphibian taxa (Džukić, Kalezić 2004, Wielstra, Arntzen 2012, Wielstra *et al.* 2013); (2) the presence of old phy-

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logenetic lineages based on mtDNA data (*e.g*. in the alpine newt, Sotiropoulos *et al.* 2007); (3) the possibility of origin and speciation in this area of many amphibians (*e.g.* crested newts, Crnobrnja-Isailović *et al.* 1997, Arntzen *et al.* 2007, Wielstra *et al.* 2013); (4) the numerous examples of peripheral segments of amphibian habitat (*e.g*. the alpine salamander, Džukić *et al.* 1997, and spadefoot toads, Džukić *et al.* 2005); (5) the presence of relict populations in some parts of Serbia (*e.g.* spadefoot toads, Džukić *et al.* 2005); and (6) the presence of populations with facultative paedomorphosis (smooth newts, Rot-Nikčević *et al.* 2000).

On considering the issues of the species conservation, many factors should be taken into account. Generally, two groups of these factors are the most relevant: (1) threats, which can be general and/or species-specific, and (2) the species biological characteristics. The first group of threats may be related to the road-killing due to vehicular traffic, especially during amphibian migrations (see Hels, Buchwald 2001). Furthermore, harvesting (Dodd 1997), along with the exceptionally high level of destruction of species habitats (Cushman 2006), are among species-specific treats. The second group includes traits related to: (1) species distribution (*e.g.* range, elevation distribution, population marginalities, endemicity); (2) ecology (*e.g*. number of habitat types, population dynamics in overall increase or decrease in the population over time, breeding success rates); (3) life history (*e.g.* reproductive mode, number of eggs or offsprings, longevity). The genetic characteristics (*e.g*. the level of genetic variability in populations and that of genetic substructuring between populations) can be relevant to the conservation issues.

Our purpose in this paper was to consider the conservation issues based on various phenotypic datasets, which include distributional, life history and ecological data. We also aimed at evaluating the differences among the general biological characteristics of the species, which are the most relevant for conservation issues.

Material and Methods

In respect to taxonomy and nomenclature issues, we followed the suggestions of Speybroeck *et al.* (2010). With regard to the parameters used, we basically followed the approach and improvements of Andreone, Luiselli (2000), with adding new parameters (PP – geographically peripheral populations; AI – species-specific direct anthropogenic influence; FR – frequency of reproduction; LC – polyphenism in life cycle of members of the Urodela due to facultative paedomorphosis). When possible, we used data obtained studying Serbian amphibians. However, in other cases we used the data obtained from abroad conspecific populations. In total, 10% of the data used in this study are from literature (see Appendix 1). In terms of the life history traits, 68% of the data about the number of eggs and 76% of those about the maximal age were taken from the literature.

Description and categories of the variable

Each variable was classified into several categories with scores ranging from the least risk (value 1) to the highest risk (maximal score value different for each variable, ranging from 2 to 5). These variables are presented below.

Distribution data

Analyses of the distributional characteristics were based on the established amphibian distributions in Serbia according to Vukov *et al.* (2013).

Distribution breadth of occupancy (DB): 1 = present in > 50% of the country; 2 = present in 10-50% of the country; 3 = present in < 5% of the country (whole taxon distribution area was calculated from the IUCN Red List distribution maps of threatened species, www.iucnredlist.org).

Endemicity (E): 1 = 0-10% of the whole taxon distribution occurs in Serbia; 2 = 10-50% of the whole taxon distribution occurs in Serbia; 3 = 50-80% of the whole taxon distribution occurs in Serbia.

Elevation distribution (ED): 1 = ubiquitous (0-2000 m); 2 = present only at high altitudes (>1000 m); 3 = present on hills and land forms up to 1000 m; 4 = restricted to plains (< 200 m elevation). The taxa that live at low altitudes are more exposed to a set of anthropogenic impacts, such as chemical, thermal, and organic pollution, road traffic, habitat alteration and destruction, and introduction of exotic species (Andreone, Luiselli 2000).

Area fragmentation (AF): 1 = fragmentation absent; 2 = fragmentation present. The fragmentation and consequent isolation of populations prevent biotic exchanges and in this way affect the population survivorship and diversity: the more fragmented is the habitat (and original species distribution area), the more isolated and exposed to local extinctions are the populations (Andreone, Luiselli 2000). The evaluation of the area fragmentation was done by using the distribution area maps for Serbia from Vukov *et al.* (2013). If there is no continuality in the established distribution of species, we considered it as fragmented.

Geographically peripheral populations (PP): 1 = absent; 2 = present. In comparison to core populations, the peripheral populations show lower levels of genetic diversity, higher risk of inbreeding and genetic drift (*e.g.* Peterman *et al.* 2013). If the species distribution area margins are on the territory of Serbia, we considered this species with peripheral populations.

Ecological data

Habitat breadth (HB): 1 = species found in all four climatic zones; 2 = species found in three climatic zones; 3 = species found in two climatic zones; 4 = species found in a single climatic zone. This parameter is based on the occurrence in the four climatic zones of Serbia (Stevanović, Stevanović 1995; modified: 1 = (sub)Mediterranean climate type; 2 = temperate-continental climate type; 3 = continental climate type; 4 = mountain climate type). This variable reflects a tendency towards euryoky of the species.

Ecology habits (HT): 1 = species with diurnal secretive activity; 2 = species with fossorial plus nocturnal or aquatic activity; 3 = species with above ground plus nocturnal activity; 4 = species with diurnal and apparent above ground activity. This parameter is categorised on the basis of the type of general phenology exhibited by amphibians in their natural environment. The variable appears less informative, but we assume that the secretive species are in general less affected by direct impact, such as predation or collection for food, pets, etc.

Aquatic habitat (for reproduction) (AHR): 1 = no free larval phase; 2 = taxon that utilises small temporary and large permanent water bodies; 3 = taxon that utilises large permanent water bodies; 4 = taxon that utilises oxygenated water bodies or slow running streams; 5 = taxon that utilises small temporary water bodies.

Adaptability to altered environments (AH): 1 = extremely adaptable species (found in urban centres); 2 = adaptable species (besides in urban habitats, found in suburbs if small natural fields are available); 3 = less adaptable species (found in average sized (~50 ha) natural woodlands at best); 4 = virtually inadaptable species (found only in large patches of habitats with natural vegetation). This trait is categorised on the basis of the personal experience of the authors (Georg Džukić and Miloš L. Kalezić, in the period 1975-2012), and/or data available in the literature.

Species-specific direct anthropogenic impact (harvesting and road killing) (AI): 1 = absent; 2 = present.

Life history data

Reproduction mode (RM): 1 = oviparity and viviparity; 2 = oviparity only; 3 = viviparity only.

Frequency of reproduction (FR): 1 = taxon with a single reproduction event per year; 2 = taxon, in which the reproduction does not occur yearly (Günther, Grossenbacher 1996). We assume that a taxon, which breeds several times a year, may recover more easily in case of habitat alterations.

Number of eggs (offspring) (EN): 1 = > 200 eggs/newborns; 2 = 50-200 eggs/newborns; 3 = < 10 eggs/newborns.

Maximum age (MA): 1 = species with maximum age of >15 years; 2 = species with maximum age of 11-15 years; 3 = species with maximum age of 6-10 years. The most data about longevity came from skeletochronological studies, and a few from observations of animals kept in captivity (*Triturus carnifex,* Cvetković *et al.* 1996).

Polyphenism in life cycle of the Urodela due to facultative paedomorphosis (coexistance of two morphs within the same population – gilled aquatic paedomorphs and terrestrial metamorphs, LC): 1 = absent; 2 = present. Populations exhibiting facultative paedomorphosis warrants preservation in order to protect this unique evolutionary process due to its impacts on species morphology, life history, genetics and evolution (*e.g.* Denoël *et al.* 2005).

Statistical procedures

In order to estimate the level of the relative threat for each species of amphibians in Serbia, the mean scores for the variables were used. These scores were calculated separately for each group of variables (distribution, ecology, and life history), and overall for all variables. Prior to the calculation of the mean scores, those for each variable were standardised to values from zero to one. The mean scores closer to one were assumed to imply higher risk for that species.

Dissimilarity distance matrix among species, based on the different scores for each group of variables (distribution, ecology, and life history), was used as input data for a Principal Coordinate Analysis (PCoA) for revealing the similarity pattern of distribution, ecology and life history characteristics among the analysed amphibian species. The PCoA computations were done by using the function "PCoA" from the "ape" library (Paradis *et al.* 2004) of the R statistical language (R Development Core Team 2006).

Results

Urodeles

The scores for the analysed variables in the eight urodele taxa in Serbia are presented in Table 1.

Overall, the mean values of the scores in all tested variables range from 0.53 for *L. vulgaris* to 0.71 for *S. atra* and 0.73 for *T. dobrogicus* (Fig. 1). *Triturus* species and *S. atra* have higher mean scores in comparison to the other species (*I. alpestris*, *L. vulgaris,* and *S. salamandra*) (Fig. 1). The mean scores of each group of variables (distribution, ecology, and life history) show that high scores of distribution and ecology variables contribute the most to the overall scores per species (Fig. 2). Only for *S. atra* and *L. vulgaris,* the life history has more important impact on their overall score. Regarding the species with the highest overall scores (*S. atra* and *T. dobrogicus*), the most important contribution to the overall score is given: in *S. atra* by some distinctive life history characteristics (viviparous species with small number of newborns), and in *T. dobrogicus* by the distribution characteristics (area restricted to plains, <200 m) (Table 1).

In general, the PCoA based on the Urodela species scores for the distribution variables separates the species with higher scores from those with lower scores along the first axis (Fig. 3). In more details, the first axis (42.7% of the variance) clusters in the cline-like manner species with fragmented area and with the present peripheral populations (*T. cristatus*, *T. dobrogicus*, *T. ivanbureschi, I. alpestris,* and *S. salamandra*), resulted from the species with non-fragmented area but with peripheral populations to the species with non-fragmented area and without peripheral populations (*L. vulgaris*) (Fig. 3, Table 1). The second axis (26.8% of the variance) does not cluster species in a clear manner, but it can be noticed that the species with distribution area of 10-50% of the Serbian territory are on the one side (*e.g. T. dobrogicus* and *T. macedonicus*), while the species with very small (*T. cristatus* and *S. atra*), or very large distribution areas (*L. vulgaris* and *I. alpestris*) in Serbia are on the other side of the spectrum described by the second axis (Fig. 3, Table 1).

The PCoA based on the species scores for the ecological variables clusters along the first axis (41.1% of the variance) all newts on the one side and salamanders on the other side (Fig. 4). The second axis (22.1% of the variance) separates the species with the highest overall score (*T. dobrogicus* and *T. cristatus*) from the other newts, as well as two salamander species (Fig. 4).

The PCoA based on the species scores for the life history variables separates the species with higher scores from those with lower scores along the second axis (Fig. 5). *Salamandra atra* is separated from the rest of the species along the first axis (49.3% of the variance) (Table 1). *Triturus dobrogicus* and *L. vulgaris* are separated from the other newts along the second axis (25.3% of the variance) because they reach a low maximal age (Table 1).

Anurans

The scores for the analysed variables in the 13 anurans species of Serbia are presented in Table 1. Overall, the values of the mean scores for all tested variables ranged from 0.54 in *H. arborea, B. bufo, P. ridibundus*, and *R. dalmatina*, up to 0.77 in *P. lessonae* and *P. syriacus* (Fig. 6). As a whole, *B. bombina*, *P. lessonae*, *P.* kl. *esculentus, R. graeca*, *P. fuscus*, and *P. syriacus* have higher overall scores than the other anuran species. The species with the highest overall scores, *R. lessonae*, has high scores for all three groups of variables, while in *P. syriacus* the overall

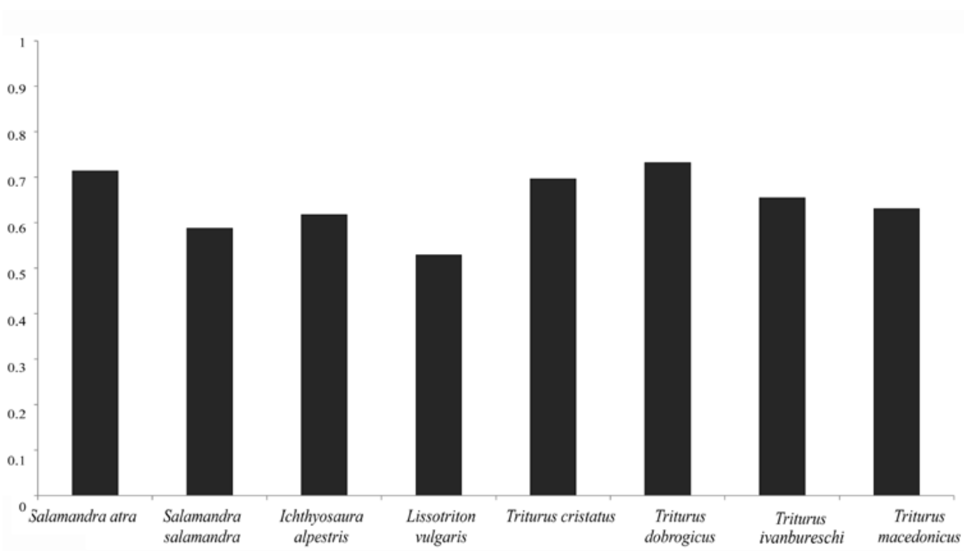

Fig. 1. Overall mean scores for the variables related to the conservation status of the Serbian caudate amphibian fauna

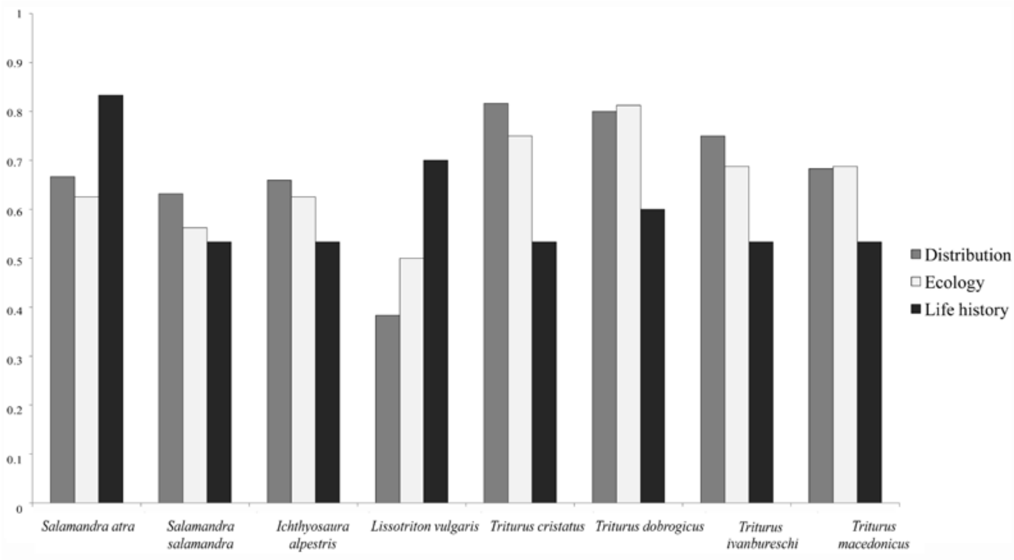

Fig. 2. The mean scores for the distribution, ecology and life history variables related to the conservation status of the Serbian caudate amphibian fauna

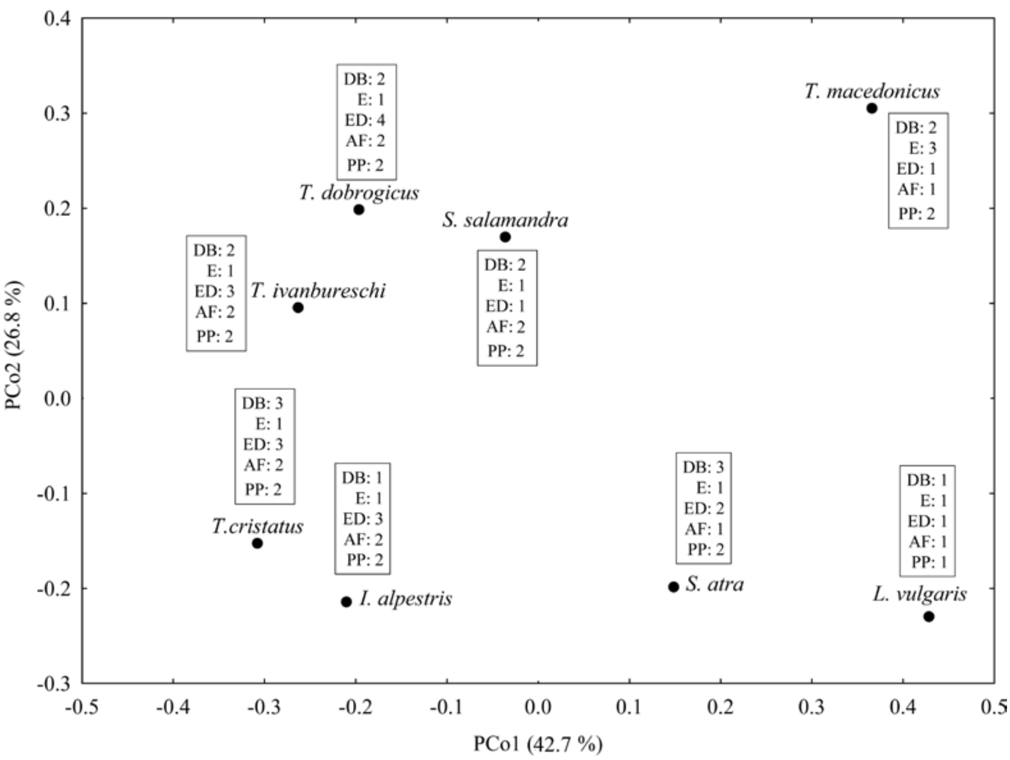

Fig. 3. Principal coordinate analysis of eight caudate amphibian species that inhabit Serbia, based on dissimilarity matrix for distribution variables. Abbreviations of variables are given in the Material and Methods section

score is under the strong influence of the distribution characteristics, such as area restricted to planes < 200 m and low distribution occupancy (Table 1). The mean scores for each group of variables (distribution, ecology, and life history) shows that high ecology variable scores contribute the most to the overall scores per species. In some species (*e.g*. *B. bombina* and *B. variegata*), along with the ecology characteristics, the distribution variable scores have important additional impact on the overall score for the species. The life history variable scores are exceptionally important for the conservation status assessment in *H. arborea*, *B. viridis*, and *R. dalmatina* (Fig. 7).

In general, the PCoA based on the species scores for the distribution variables separates species with higher scores from those with lower scores along the first axis (Fig. 8). In more details, the first

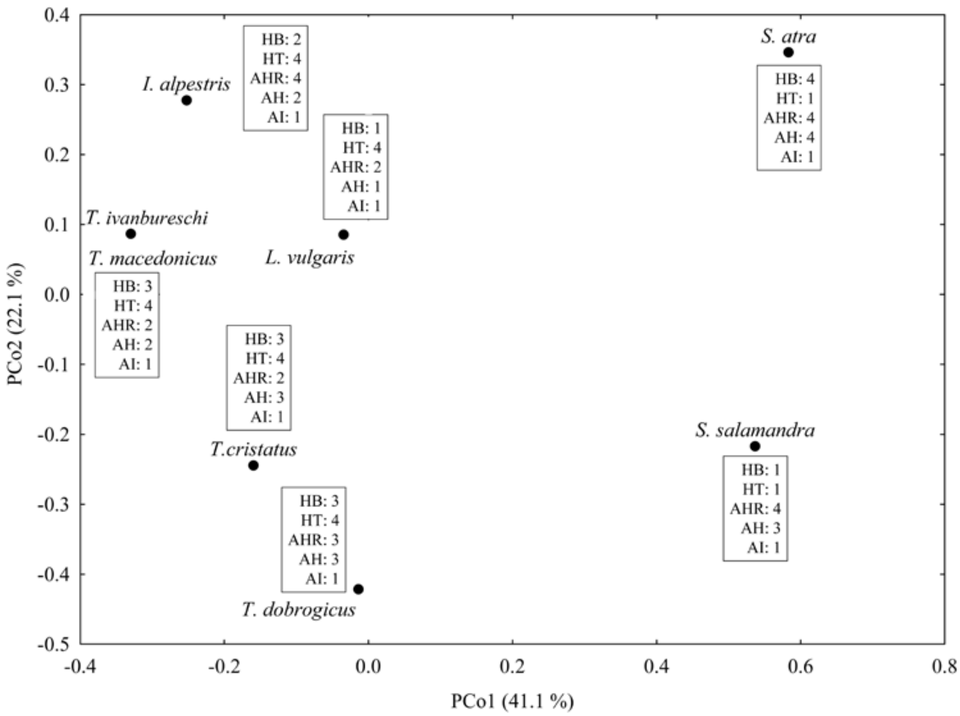

Fig. 4. Principal coordinate analysis of eight caudate amphibian species that inhabit Serbia based on dissimilarity matrix for ecology variables. Abbreviations of variables are given in the Material and Methods section

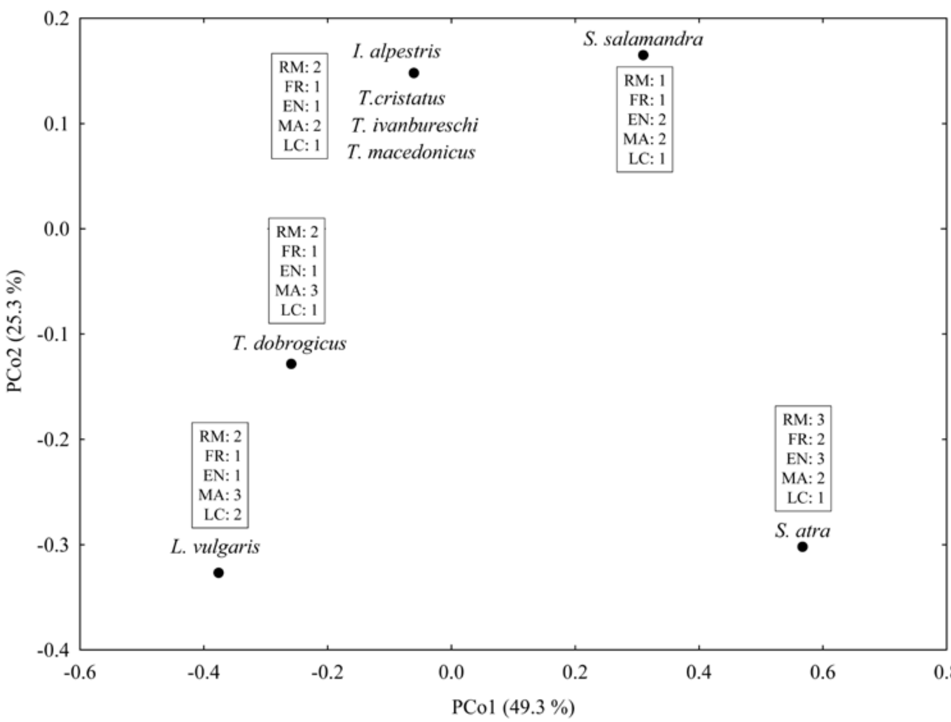

Fig. 5. Principal coordinate analysis of eight caudate amphibian species that inhabit Serbia based on dissimilarity matrix for life history variables. Abbreviations of variables are given in the Material and Methods section

axis (51.2% of the variance) separates the species with low distribution occupancy in Serbia (*e.g. P. syriacus*, *P. fuscus*, *B. bombina,* and *B. variegata*) from the widely distributed species (*e.g. H. arborea, B. bufo, B. viridis* and *R. dalmatina*) (Table 1). The second axis (19.2% of the variance) separates *R. graeca* from all other anuran species because the former has some level of endemicity (10±50% of the whole taxon distribution is in Serbia) (Fig. 8, Table 1).

Table 1. Scores for the variables related to the conservation status of the Serbian amphibian fauna

	Distribution					Ecology					Life history					
	1. DB	**2. E**	**3. ED**	**4. AF**	**5. PP**	**6. HB**	**7. HT**	**8. AHR**	**9. AH**	**10. AI**	**11. RM**	**12. FR**	**13. EN**	**14. MA**	**15. LC**	**Mean score**
Caudate amphibians																
Salamandra atra	3	1	2	1	2	4	1	1	4	1	3	2	3	2	1	0.71
Salamandra salamandra	2	1	1	2	2	1	1	4	3	1	1	1	2	2	1	0.58
Ichthyosaura alpestris	1	1	3	2	2	2	4	2	2	1	2	1	1	2	1	0.61
Lissotriton vulgaris	1	1	1	1	1	1	4	2	1	1	2	1	1	3	2	0.53
Triturus cristatus	3	1	3	2	2	3	4	2	3	1	2	1	1	2	1	0.70
Triturus dobrogicus	2	1	4	2	2	3	4	3	3	1	2	1	1	3	1	0.73
Triturus ivanbureschi	2	1	3	2	2	3	4	2	2	1	2	1	1	2	1	0.65
Triturus macedonicus	2	3	1	1	2	3	4	2	2	1	2	1	1	2	1	0.63
Anuran amphibians																
Hyla arborea	1	1	1	1	1	1	4	2	3	1	2	1	1	3	1	0.55
Bombina bombina	2	1	4	1	2	3	1	3	4	1	2	1	1	2	1	0.68
Bombina variegata	1	1	3	2	2	1	1	5	2	1	2	1	2	1	1	0.63
Bufo bufo	1	1	1	1	1	1	3	2	3	2	2	1	1	2	1	0.54
Bufo viridis	1	1	3	1	1	1	3	2	1	2	2	1	1	3	1	0.57
Pelophylax lessonae	2	1	4	1	2	3	4	3	2	2	2	1	1	3	1	0.77
Pelophylax kl. esculentus	1	1	3	1	2	3	4	3	1	2	2	1	1	2	1	0.68
Pelophylax ridibundus	1	1	1	1	1	1	4	3	1	2	2	1	1	2	1	0.54
Rana graeca	2	2	3	1	2	2	4	4	4	1	2	1	1	1	1	0.73
Rana dalmatina	1	1	1	1	1	1	4	2	3	1	2	1	1	3	1	0.55
Rana temporaria	1	1	2	2	2	2	4	2	2	1	2	1	1	2	1	0.63
Pelobates fuscus	2	1	4	2	2	3	2	2	4	2	2	1	1	1	1	0.74
Pelobates syriacus	3	1	4	2	2	3	2	2	4	2	2	1	1	1	1	0.77

The PCoA based on the species scores for the ecology variables clusters along the first axis (26.7% of the variance) the anuran species according to climatic zones they inhabit and presence or absence of anthropogenic influence (*e.g. P. fuscus, P. syriacus, P.* kl. *esculentus,* and *P. lessonae* inhabit two zones and they are under anthropogenic influence) (Fig. 9). The second axis (22.5% of the variance) separates *B. bufo* and *B. viridis* according to their species-specific above ground nocturnal activity (Table 1).

The PCoA based on the species scores for the life history variables clusters the anuran species in four main groups along the first and second axes (46.4% and 40.8% of the variance, respectively) (Fig. 10). It should be noted that only two life history variables (the egg number and maximal age) show variation, while the other three are uniform, and therefore, uninformative for the separation of the species groups. Some species with high overall score, such as *P. fuscus* and *P. syriacus,* show very low score for the life history variables (Table 1), which means that the life history traits do not have important impact on their overall scores.

Discussion

The results of our analyses showed that in urodeles the high scores of the distribution and ecology variables contribute the most to the overall scores per species. In anurans, the high scores of the ecology variable contribute the most to the overall scores. It seems that in both groups, the life-history variables had low contribution to the total scores for the species.

In regard to the caudate amphibians, according to our results, *S. atra* and *T. dobrogicus* are the most sensitive to factors which cause decline. *Salamandra atra* is recognised as Least Concerned on the IUCN Red List, while *T. dobrogicus* is rated as Near Threatened species closer to being qualified as Vulnerable in the near future (see also Arntzen *et al.* 1997). The alpine salamander (*S. atra*) is characterised by specific ecological requirements and peculiar life-history traits, as well as by one of the lowest level of genetic differentiation and an extremely high level of population density, at least in some parts of the distribution range (Helfer *et al.* 2012).

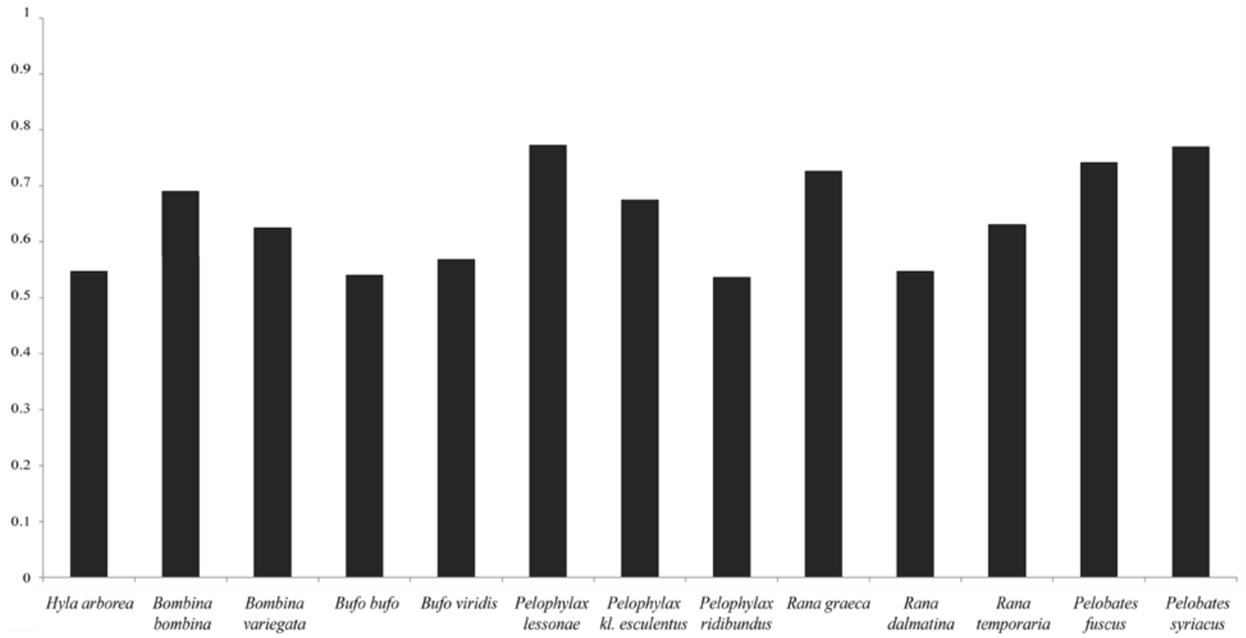

Fig. 6. Overall mean scores for the variables related to the conservation status of the Serbian anuran amphibian fauna

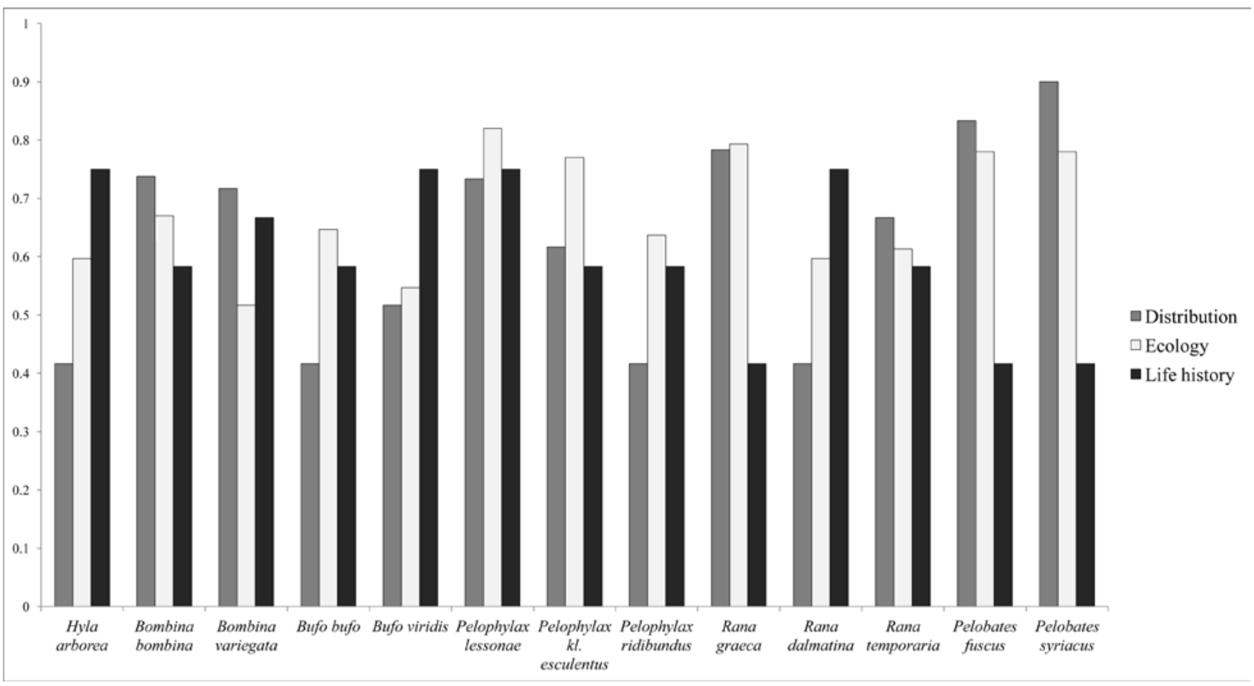

Fig. 7. Overall mean scores for the variables related to the conservation status of the Serbian anuran amphibian fauna.

However, because of the exceptionally small range of this species in Serbia, and its high vulnerability to external threats (such as localised habitat destruction in combination with very limited adaptability), we consider the species as endangered in Serbia. In comparison with the other Serbian caudate amphibians, *T. dobrogicus* occurs in lowlands within uniform habitats, which make this crested newt more sensitive to rapid habitat loss, due to agricultural development and intensive hydromelioration, than the other amphibians (Andrejev 2004, Krizmanić 2008). Therefore, we evaluate this species particularly vulnerable for Serbia. In favour of its survival is the exceptionally low level of genetic substructuring (*e.g.* high level of gene flow among populations) (Vörös, Arntzen 2010). In respect of the biological characteristics analysed in this study, the other crested newt species in Serbia (*T. cristatus*, *T. ivanbureschi,* and *T. macedonicus* in decreasing order) can be considered as relatively vulnerable, in particular,

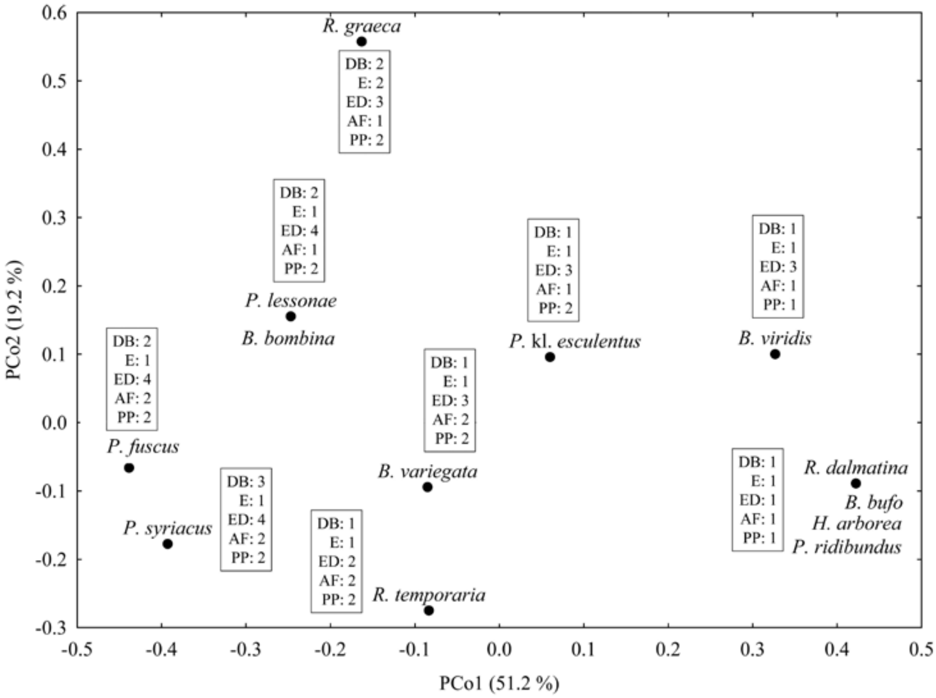

Fig. 8. Principal coordinate analysis of 13 anuran amphibian species that inhabit Serbia, based on dissimilarity matrix for distribution variables. Abbreviations of variables are given in the Material and Methods section

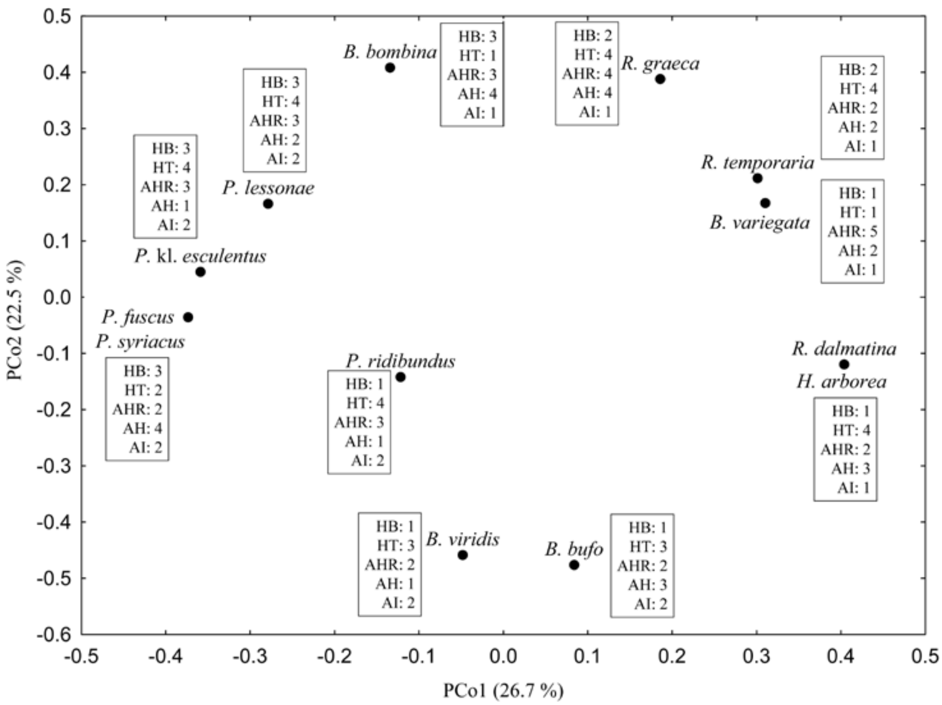

Fig. 9. Principal coordinate analysis of 13 anuran amphibian species that inhabit Serbia, based on dissimilarity matrix for ecology variables. Abbreviations of variables are given in the Material and Methods section

because of the relatively fast loss of their habitats (Crnobrnja-Isailović *et al.* 2005) in comparison with the other European newts (*I. alpestris* and *L. vulgaris*). According to the values of the biological characteristics used in this study, the other Urodela species (*I. alpestris, L. vulgaris,* and *S. salamandra*) seems not to be in particular danger in Serbia.

On the grounds of the distributional and ecological traits, the two fossorial species (*P. fuscus* and *P. syriacus*), one of the green frogs (*R. lessonae*), one of the brown frogs (*R. graeca*, the only anuran species endemic for the Balkans), as well as *B. bombina*

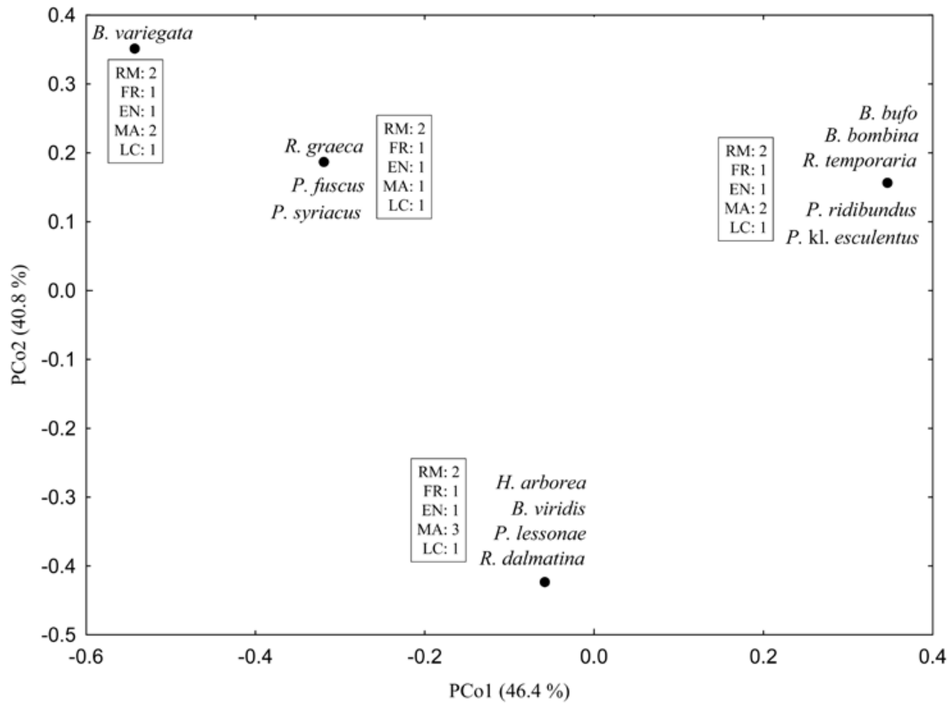

Fig. 10. Principal coordinate analysis of 13 anuran amphibian species that inhabit Serbia, based on dissimilarity matrix for life history variables. Abbreviations of variables are given in the Material and Methods section

(a virtually inadaptable species) may be considered as potentially the most vulnerable to external threats among the anurans in Serbia.

The remaining Serbian anuran amphibians (*H. arborea*, *B. bufo*, *B. viridis*, *B. variegata*, *R. dalmatina*, *P.* kl. *esculentus,* and *P. ridibundus*) are apparently not endangered.

Our results give the general overview of the conservation issues on the basis of distribution, ecological and life-history data. However, we have to point out the presence of specific risks in some species, which must be taken into account in preparing conservation measures and management plans in Serbia. For example, the group of the green frogs are additionally threatened by the long-lasting commercial harvesting, which still occurs in Serbia in spite of the legal regulations (Ljubisavljević *et al.* 2003). In this respect, the threat is higher for *P. ridibundus* because of its larger size in comparison to the other green frog species in the country.

The indirect influences of habitat destruction were not tested because there were no enough data to quantify the impact of the habitat loss on the amphibians in Serbia. However, it seems obvious that many species, such as *B. bombina*, *T. dobrogicus* and *R. lessonae*, are distributed in the parts of the country under high and long-lasting anthropogenic influences (agriculture, habitat destruction, melioration, etc.) and those species are highly vulnerable to the constant and growing environmental threats (this study).

In conclusion, it must be noted that due to lack of many relevant data, we are still far from developing a realistic view on the conservation status of Serbian amphibians. There are no large-scale and long-lasting population studies to provide valuable data on the population dynamics, particularly in the species that are in risk. However, this study gave us at least some information which species are the most endangered.

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Appendix 1

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