Morphometrics of the Yellow-bellied Toad (*Bombina variegata*) in the Central Balkans: Implications for Taxonomy and Zoogeography

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Tanja D. Vukov, Georg Džukić, Suvad Lelo, Leo J. Borkin, Spartak N. Litvinchuk, and Miloš L. Kalezić (2006) Morphometrics of the yellow-bellied toad (*Bombina variegata*) in the Central Balkans: implications for taxonomy and zoogeography. *Zoological Studies* **45**(2): 213-222. A comprehensive survey of the Central Balkan yellow-bellied toad (*Bombina variegata*) populations was undertaken to describe morphometric differentiation between 4 geographic groups previously demarcated by molecular studies. Our analyses confirmed pronounced geographic structuring at the population level, as well as a clear transition over a relatively short distance separating the localized *B. v. scabra*, endemic to the Balkans, and the widely distributed *B. v. variegata*. We present the inferred range boundaries of these geographic groups in the Central Balkans, and discuss their historical zoogeography and taxonomy. Our study provides evidence that the Northern Balkans, a secondary transition zone of postglacial origin, constitutes an important European suture area for amphibians.
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Key words: *Bombina variegata*, *Bombina variegata scabra*, Morphometric differentiation, Taxonomy, Zoogeography.

The yellow-bellied toad, *Bombina variegata* (Linnaeus, 1758), inhabits higher elevations of western Europe, the Balkan Peninsula, and the Carpathian Mountains, as well as isolated ridges of the Hungarian Plain (e.g., Arntzen 1978, Gollmann et al. 1997). Traditionally regarded as polytypic, this species is represented by 4 subspecies. The nominotypical subspecies extend from France to the Carpathian Mountains. The Italian taxon, *B. v. pachypus* (Bonaparte, 1838), is confined to the Apennines south of the Po River valley, and *B. v. scabra* (Küster, 1843) is found in the Balkans (e.g., Gollmann et al. 1997, Arnold and Ovenden 2002). A separate taxonomic status for populations that are found in a restricted area of the Dalmatian coast, classified earlier as *B. v. kolombatovici* (Bedriaga, 1890), has been disputed. A recent analysis suggests that *B. v. kolombatovici* is identical to the nominal subspecies (Fromhage et al. 2004), however, this assumption is based on a single mtDNA sequence and further analyses are necessary. If the allopatric Italian taxon is excluded, the range of the yellow-bellied toad can be subdivided into 4 geographic groups on the grounds of distinct allozyme and/or mtDNA haplotype variation patterns (e.g., Szymura 1988 1993, Szymura et al. 2000, Radojičić 2000, Fromhage et al. 2004). Within the phenotypic characteristics of *B. v. variegata*, Western European and Carpathian (herein referred to as

eastern) groups can be distinguished. According to Fromhage et al. (2004), the *scabra* phenotype is presumably differentiated into southern and northern lineages. The ranges of these currently recognized geographic groups come into contact in the Central Balkans. Geographic differentiation in this region seems substantially greater than elsewhere and is characterized by a transition zone with gradual clinal variations (Szymura 1993, Szymura et al. 2000). Thus, the central Balkans and adjacent areas (e.g., the Carpathian massif) represent the core region of the geographic range of the yellow-bellied toad in terms of evolutionary interest.

Surprisingly, in spite of the relatively extensive research of the genetic diversity of yellow-bellied toad populations, including allozyme and mtDNA variations, no studies have examined the morpho-

logical characteristics of genetically distinct geographic groups. Whether distinct haplotype clades determine separately evolving lineages or represent gene lineages that have evolved within a single organism lineage can be determined by comparing morphological and genetic variations.

In this study, we concentrated on the morphometric differentiation of *B. variegata* in the central Balkan Peninsula. The main objective of this work was to study the patterns of morphometric variations within and among genetically distinct geographic groups. Additional goals were to present the inferred range boundaries of the geographic groups found in the Central Balkans and provide insights into some relevant issues of *B. variegata* evolution in the Balkans and its taxonomy.
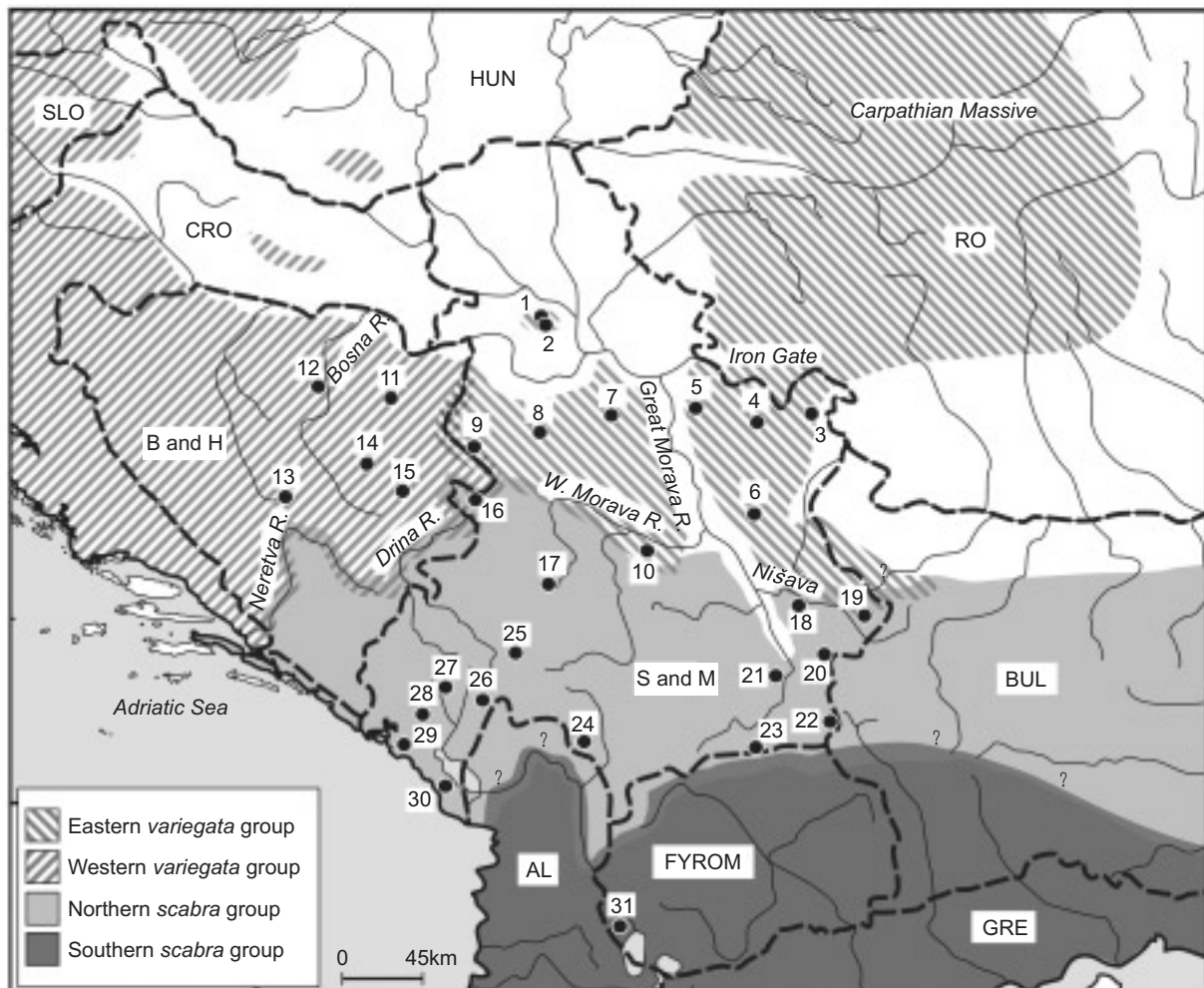

Fig. 1. Distribution of morphologically and genetically distinct geographical groups of the yellow-bellied toad in the Central Balkan inferred from taxonomical assessments of analyzed population samples (allozyme and mt DNA studies: Szymura 1993, Szymura et al. 2000, Radojičić 2000, Fromhage et al. 2004; morphometric study: this paper). The positions of the studied population samples are designated by numbers (see the Appendix for location data).

MATERIALS AND METHODS

Population samples

Yellow-bellied toads were collected from 31 populations (Fig. 1). The locality data, i.e. the name, elevation, UTM code (10 x 10 km square), and the numbers of females and males collected are listed in the Appendix.

In total, 631 sexually mature, ethanol-preserved individuals that were collected during the breeding season were analyzed. Adult males were recognized by the presence of nuptial pads. Females attain sexual maturity when they reach a minimal size of 34 mm (Barandun et al. 1997). Thus, all toads larger than 34 mm without nuptial pads were recognized as adult females.

Multivariate morphometrics

Since many morphometric characters of the yellow-bellied toad demonstrate significant sexual dimorphism (Radojičić et al. 2002), analyses were performed separately for females and males. The morphometric variability of 20 characters was studied. Descriptions of the characters are presented in the Appendix. To avoid observer bias, the 1st author took all measurements with a digital caliper to a precision of 0.01 mm.

ANOVA and MANOVA tests with sex, population, and subspecific taxon (*variegata* and *scabra*) as sources of variability were used to test for differences between the mean values of the morphometric traits of population samples.

The log-transformed and standardized (0 mean, 1 variance) dataset was subjected to principal component analysis (PCA) to explore group-related size patterns. Therefore, the allometric relationship between PC 1 values (individual scores on the 1st component used as a latent size variable) and measures of individual size (L values) were examined. Slopes and intercepts of the regression lines describing the size-PC 1 relationships were compared using the ANCOVA test.

To reduce the impact of overall size, the standardized residuals of the regression log <character

Table 1. Descriptive statistics of 20 body measurements of adult males (♂) and females (♀). Sample size (*n*), mean value (in mm) ± standard error (SE); statistical significance of differences between subspecies tested by ANOVA (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns, non-significant). Abbreviations of characters are given in the Appendix

| | *B. v. variegata* | | | | *B. v. scabra* | | | | *B. v. variegata* *B. v. scabra* | |
| | ♂ (*n* = 181) | | ♀ (*n* = 97) | | ♂ (*n* = 213) | | ♀ (*n* = 140) | | ♂ | ♀ |
Character	mean	SE	mean	SE	mean	SE	mean	SE	P	P
L	43.07	0.26	44.72	0.38	43.64	0.31	44.61	0.42	ns	ns
F	16.58	0.11	16.15	0.15	17.09	0.13	16.54	0.14	**	ns
T	15.98	0.09	15.37	0.10	16.19	0.10	15.37	0.11	ns	ns
N	26.17	0.14	25.71	0.17	26.82	0.18	25.84	0.20	*	ns
P	16.52	0.10	16.31	0.12	17.06	0.13	16.50	0.14	**	ns
H	10.16	0.08	8.95	0.08	10.48	0.09	9.04	0.08	*	ns
M	16.15	0.09	15.98	0.11	16.96	0.11	16.56	0.13	***	**
DpPa	4.13	0.03	4.07	0.03	4.60	0.04	4.52	0.04	***	***
DsPa	6.05	0.04	6.06	0.04	6.62	0.05	6.55	0.06	***	***
DpPp	3.87	0.03	3.81	0.04	4.29	0.04	4.20	0.05	***	***
Cint	1.22	0.01	1.20	0.01	1.36	0.02	1.35	0.02	***	***
Lc	10.29	0.05	10.34	0.06	10.16	0.07	10.02	0.07	ns	***
Ltc	13.34	0.07	13.37	0.09	13.53	0.09	13.30	0.10	ns	ns
Spp	2.81	0.02	2.87	0.03	2.78	0.02	2.70	0.03	ns	***
Spi	2.57	0.02	2.55	0.02	2.59	0.02	2.51	0.02	ns	ns
Spcr	5.95	0.03	5.87	0.04	6.08	0.04	6.01	0.05	*	ns
Lo	3.57	0.03	3.58	0.03	3.58	0.03	3.60	0.03	ns	ns
Ltp	2.62	0.02	2.60	0.03	2.72	0.02	2.72	0.02	***	**
Dro	4.92	0.03	4.83	0.03	5.01	0.03	4.91	0.04	ns	ns
Dno	3.06	0.02	3.03	0.02	3.14	0.02	3.06	0.03	*	ns

on log body length (L) were calculated and subjected to another PCA. This PCA using size-adjusted values was performed to explore morphometric variability independent of the geographic group assignment. Factor scores obtained in the PCA represented the input data for a canonical discriminant analysis (CDA). The CDA was applied to maximize differences between population samples within and among the predefined groups, i.e., the western and eastern *variegata* groups and the 2 Balkan geographic groups (southern *scabra* and northern *scabra* populations). Standardized coefficients of the canonical variables were examined in order to obtain an estimate of their respective contributions to the general morphometric variability and separation of the taxa populations.

As differences in sex-specific variability could be the consequence of differences in allometry, a bivariate allometric analysis was separately performed on females and males of the *variegata* and *scabra* samples. The allometric analysis was based on a linear regression of the log-transformed data on the log-transformed total body length (L). Homogeneity of the slope test was performed to check for differences between the allometric coefficients of the different genders.

All analyses were performed with the STATISTICA 6.0 (Statsoft 2001) computer software.

RESULTS

Significant variations between the sexes and samples (MANOVA, Wilks' lambda = 0.30, df1 = 560, df2 = 8617, p = 0.0038) were observed. A comparison of the regression lines demonstrated that the relationship between PC 1 and the total body length did not significantly differ between the geographic groups (ANCOVA test; intercepts p > 0.05, slopes p > 0.05 for both sexes), indicating an absence of different group-related size patterns. However, when the univariate analysis of size differences between *scabra* and *variegata* individuals was performed, all 12 significantly different characters of males had higher mean values in the *scabra* than *variegata* specimens, whereas such a trend was observed for six of 8 significantly different traits among females (ANOVA test, Table 1). Also, individuals from the western *variegata* group had higher mean values for most of the significantly different character values in comparison with specimens from the eastern *variegata* group (data are available upon request).

In the PCA using size-adjusted values, 4 functions in males and 5 functions in females were derived (the constraint was an eigenvalue > 1) with the last function explaining 6.83% and 6.78% of the variance for males and females respectively. The first 3 principal components explained 55.66% of the total male and 51.35% of the total female variability. The greatest factor loading values on the 1st and 2nd principal components had almost identical characters for both males and females. On the 1st principal component, several limb characteristics had the highest loading values (males: N, P, M, DpPa, DsPa, and DpPp; females: P, M, DpPa, DsPa, and DpPp), and on the 2nd, several
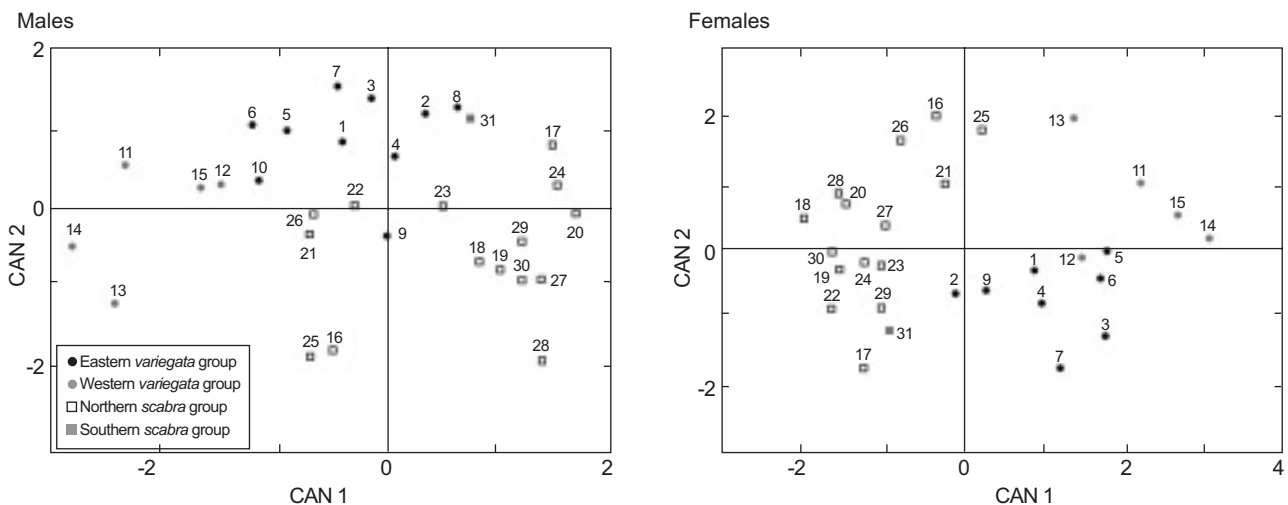

Fig. 2. Relative position of group centroids of males and females on the 1st and 2nd canonical axes (see the Appendix for location data).

characteristics of the head had the highest loading values (males: Spp, Spi, and Spcr; females: Ltc, Spp, Spi, Spcr, and Dno). Cint, Ltp, Dro, Dno for males and Ltp for females had the greatest factor loadings on the 3rd principal component (Table 2).

In the CDA, 4 significant functions in males and 5 in females were obtained. The last one explained 5.73% and 4.15% of the variance for males and females respectively. If we take into account the 1st 3 canonical axes, 94.27% of the total discrimination in males and 84.99% in females were obtained (Table 3). Ordination of individual populations along the first 2 canonical axes showed that the *variegata* taxon and *scabra* taxon were separated to the extent that there was little overlap between females and larger overlap between males (Fig. 2.; for ordination of individuals, see Fig. 3). The values of the standardized coefficients for females indicated that the greatest contributions to discrimination on the 1st canonical axis were made by the 1st and 3rd principal component axes, or more precisely, by differences in characters that had the greatest loadings on the 1st and 3rd principal component axes (i.e., P, M, DpPa, DsPa, DpPp, and Ltp, all of which had higher mean values in the *scabra* samples). The male

Table 2. Factor loadings of analyzed morphometric characters on the first 3 principal components. Abbreviations of characters are given in the Appendix

Character	Males			Females		
	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3
F	0.295	0.006	0.228	0.101	0.118	0.174
T	0.307	0.015	0.189	0.185	0.160	-0.003
N	0.573	0.064	0.079	0.466	0.184	-0.233
P	0.613	0.094	0.084	0.556	0.178	-0.237
H	0.287	0.053	0.007	0.216	-0.067	0.105
M	0.700	0.044	0.176	0.657	0.118	-0.045
DpPa	0.841	0.171	0.183	0.860	0.163	0.154
DsPa	0.859	0.156	0.165	0.834	0.194	0.124
DpPp	0.768	0.026	-0.048	0.730	0.054	-0.168
Cint	0.302	-0.169	0.574	0.493	0.114	0.406
Lc	-0.025	0.420	0.196	-0.045	0.446	-0.166
Ltc	0.086	0.332	0.441	0.020	0.570	0.298
Spp	0.114	0.825	-0.094	0.039	0.678	-0.455
Spi	0.126	0.776	0.051	0.000	0.700	-0.210
Spcr	0.097	0.579	0.483	0.295	0.662	0.048
Lo	-0.008	0.277	-0.119	0.163	-0.013	0.138
Ltp	0.031	-0.073	0.603	0.023	-0.013	0.817
Dro	0.098	0.440	0.565	0.259	0.651	0.112
Dno	0.038	0.194	0.658	0.145	0.679	0.194
Eigenvalue	6.922	2.140	1.514	6.021	2.147	1.588
Cum. Prop. (%)	36.430	47.695	55.663	31.691	42.991	51.350

Table 3. Principal component factor coefficients for the 3 canonical variates from the discriminant functional analysis. Abbreviations of characters are given in the Appendix

Factor	Males			Females		
	CAN 1	CAN 2	CAN 3	CAN 1	CAN 2	CAN 3
PC 1	0.420	-0.904	-0.031	-0.802	0.560	0.267
PC 2	-0.420	-0.170	-0.889	0.147	-0.143	0.926
PC 3	0.754	0.046	-0.011	-0.815	-0.223	-0.295
Eigenvalue	1.589	1.028	0.549	2.278	1.242	0.896
Cum. Prop. (%)	47.304	77.908	94.266	43.835	67.745	84.995

population samples of *variegata* and *scabra* were not sharply separated. However, several characters which had the greatest loadings on the first principal component axis contributed to a significant discrimination of the taxa on the 2nd canonical axis (i.e., N, P, M, DpPa, DsPa, and DpPp, all of which had higher mean values in the *scabra* sample). The same characters discriminated the *variegata* samples from the *scabra* samples more clearly on the 1st canonical axis in females and less clearly on the 2nd canonical axis in males.

The 2nd discriminant function partly separated the female *variegata* samples from Bosnia and Herzegovina (the western group) from the remaining *variegata* samples (the eastern group). Discriminant analysis showed no distinctive geographic grouping within male *variegata* samples. Within the *scabra* taxon, the only analyzed population sample belonging to the southern group (31-Podgorci) were distinguished from those of the northern *scabra* group in the males and appeared to be morphometrically closer to the *variegata* samples, whereas no such tendency was observed in females (Fig. 2).

The homogeneity of the slope test showed no significant differences between the allometric coefficients in *variegata* males and females for most of the examined characters, except for DpPa and Spp for which the coefficient values of males were greater than those of females. On the contrary, comparison of allometric coefficients between *scabra* males and females revealed significant distinctions for 10 (F, T, N, P, H, M, DpPa, DpPp, Cint, and Lc, for which females had more-pronounced allometry) of 19 characters.

DISCUSSION

Patterns of geographic variation

Some of the geographic groups previously demarcated by molecular studies were poorly sampled in our study, especially from the southern *scabra* group. Also, studying the transition zones between the *variegata* and *scabra* taxa requires closer spacing of sampling sites. Nevertheless, our analysis revealed that morphometric variation in the yellow-bellied toad from the Central Balkans is not only substantial but complex as well. Pronounced geographic structuring at the population level is involved, as well as a clear differentiation between the narrow-ranged Balkan endemic *scabra* taxon and the widely distributed *variegata* taxon. Also, samples of the *variegata* taxon in the Central Balkans form 2 morphometrically distinct geographic groups, the western and eastern distribution patterns; there is less-clear structuring within the *scabra* taxa. It is noteworthy that such patterns of geographic variation in morphometry seem to be complemented by a similar variation pattern at the molecular level (Szymura 1988; Szymura et al. 2000; Radojičić 2000; Fromhage et al. 2004).

Our study showed that differences in sex-specific variability could possibly be the consequence of differences in allometry for the *scabra* group, but not for the *variegata* group. However, the influence of sample size of different sexes on the obtained variability could not be excluded, especially for the *variegata* samples.

An intriguing issue which remains to be studied concerns the possibility that introgression of *B.*
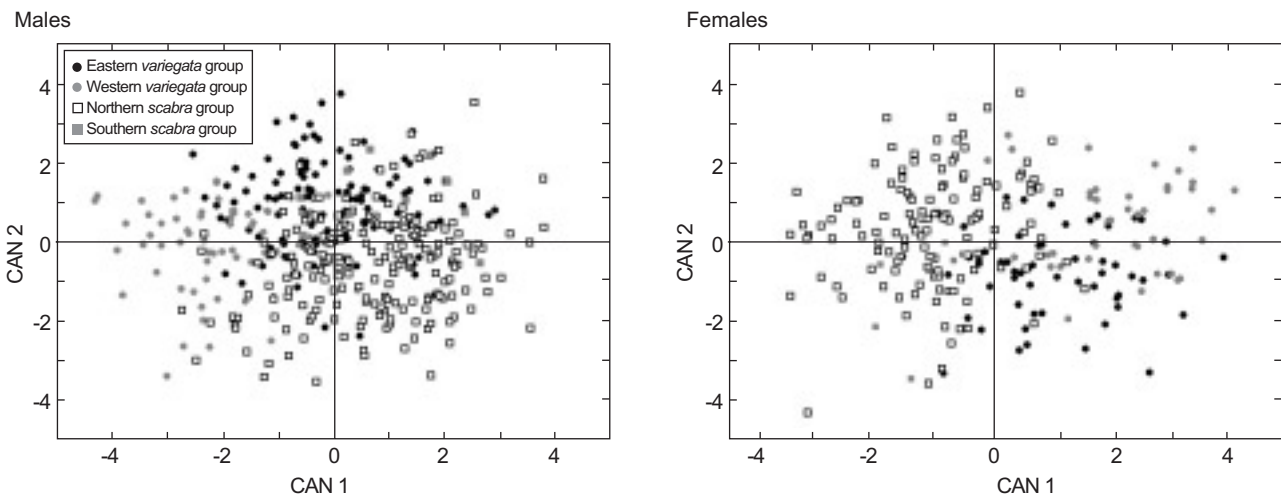

Fig. 3. Scatterplot of canonical scores for males and females of all population samples on the 1st and 2nd canonical axes.

bombina genes into local gene pools of the *B. variegata* population has contributed to the observed pattern of geographic variation in morphometry in the Central Balkan region. Namely, these 2 species have a propensity to hybridize when they come into contact, and this is true as well for populations inhabiting the basins of the Danube and Sava Rivers and their tributaries (e.g., Szymura 1993). Some of the *variegata* samples we studied (samples 1-3, and 5; Fig. 1) were obtained close to the *B. bombina* range.

At first glance, there is no zoogeographically significant barrier (e.g., a physiographic feature) capable of disrupting the gene flow either between the *variegata* and *scabra* taxa or within each taxon. Thus far, the extent of intermixing of members of the phylogeographic units of the yellow-bellied toad in the Central Balkans has not been determined. However, the presence of a transition zone of the yellow-bellied toad in the northern Balkans is also supported by clinal variations of 3 allozyme loci (Szymura 1988). Furthermore, *variegata* populations in the western and particularly in the eastern region of the Balkans are polymorphic for 3 very close mtDNA haplotypes characteristic of both the West European *variegata* group and the *scabra* taxon (Szymura et al. 2000).

The presence of a transition zone between the genetically and morphometrically differentiated geographic groups of *B. variegata*, as reported for 2 other amphibians, *Triturus vulgaris* (Kalezić 1984) and *Triturus* superspecies *cristatus* (Wallis and Arntzen 1989), suggests that the Peripannonian area of the Central Balkan region and neighboring parts of the Western Balkans form suture zones (*sensu* Remington 1968). Such zones of geographic overlap between recently joined biotic assemblages include the taxa of species and intraspecies that hybridize within a limited area.

Distribution of phylogeographic groups, their zoogeography, and taxonomy

Although precise distribution limits of the yellow-bellied groups in the Central Balkans have yet to be established, we can offer an inferred picture based on the relevant data from our morphometric study and previous genetic examinations (Szymura et al. 2000, Fromhage et al. 2004) (Fig. 1).

In the Central Balkans, a west to east oriented distribution line, roughly delineated by the Western Morava and Nišava Rivers, separates the

scabra and *variegata* ranges (Fig. 1). The population samples of the western *variegata* group that were studied were obtained from the southeastern most part of the compact yellow-bellied toad's range. On the basis of mtDNA variation data (Szymura et al. 2000, Fromhage et al. 2004), it appears that the western *variegata* group spreads along the Adriatic coast up to the Neretva River, an important zoogeographical boundary for many animal groups, including amphibians (e.g., Hadži 1935). We expected that the Great Morava River valley, by which the lowland *B. bombina* has penetrated deeply into the Balkan Peninsula (Džukić et al. 2005), would be the line of separation between the western and eastern *variegata* ranges. However, our morphometric study showed that the Drina River valley separates these groups (Fig. 1). Clearly, this issue needs to be confirmed by molecular marker studies based on appropriate sampling. The eastern *variegata* group in the Central Balkans was, at least historically, connected to populations from the Carpathian massif by the Iron Gate in a way which has been demonstrated for other amphibians (e.g., *Triturus* superspecies *cristatus*; Kalezić et al. 1997).

Despite these clear geographical patterns, the interpretation of the distributions in terms of refugia and colonization routes can only be tentative (see Arntzen 1978, Szymura 1993), as we still need to determine the phylogeny of geographic groups. Instead, there are conflicting phyletic affinities, based on discordances of the mtDNA analysis (with *scabra* and the western *variegata* being sister groups) vs. allozyme and morphological analyses (with the western and Carpathian *variegata* being sister groups; Szymura et al. 2000, this study). In view of the findings obtained from the allozyme and morphological analyses, and the observed high degree of molecular and morphological differentiation, we assumed that the *scabra* and *variegata* taxa have probably resided in their present localities for a long time (the vicariance event occurred before the Pleistocene glacial periods).

Is the degree of differentiation in the yellow-bellied toad complex in the Balkans sufficient to call into question the current delineation of the taxon borders and to elevate the taxonomic position of some groups from the subspecific to the species level? There are various criteria for diagnosing species when dealing with differences in morphology, genetic markers, and ecology (e.g., De Queiroz 1998, Puorto et al. 2001, Helbig et al. 2002). The list of these criteria includes reproductive discontinuity (or lack of intergradation in the

phenotype as a surrogate; Paterson 1985), diagnostic criteria (fixed differences in some phenotypic or genotypic characters; Mallett 1995), niche criteria (Van Valen 1976), phenetic cluster criteria (Sokal and Sneath 1973), and the exclusivity of gene coalescence (Baum and Shaw 1992). The genome sizes of the 2 main Balkan yellow-bellied toad geographic groups, *scabra* and *variegata*, considerably differ (Borkin et al. 2005). The ranges of genome size variations in these 2 taxa do not overlap. Moreover, the *scabra* genome size proved to be closer to *B. bombina* than to *variegata*. *Scabra* populations cannot readily be distinguished from *variegata* samples by the allelic composition at certain gene loci (Szymura 1988, Radojičić 2000, Borkin and Litvinchuk unpubl. data). Delineation between the *variegata* and *scabra* groups was also supported by a bioacoustic study (Vasara et al. 1991). Thus, according to currently available evidence, the *scabra* group is distinct from the more-northerly distributed *variegata* group. Thus, the lineages possibly represent 2 distinct species. More-comprehensive morphological and molecular studies with more-rigorous sampling designs are required to resolve this issue.

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APPENDIX I :

Location data

Location of populations, elevation, UTM code, and numbers of measured males and females, respectively.

S and M, Serbia and Montenegro; B and H, Bosnia and Herzegovina; FYROM, Former Yugoslav Republic of Macedonia; ♂, male; ♀, female.

Eastern *variegata* group 1. Andrevlje (S and M, 210 m, CR90, 10 ♂♂ + 10 ♀♀; 2. Kamenolom (S and M, 320 m, CR90, 11 ♂♂ + 10 ♀♀); 3. Cvetanovac (S and M, 388 m, FQ02, 15 ♂♂ + 8 ♀♀); 4. Lisaja (S and M, 560 m, 17 ♂♂ + 7 ♀♀); 5. Zaova (S and M, 199 m, EQ22, 7 ♂♂ + 7 ♀♀); 6. Krivi Vir (S and M, 419 m, EP65, 8 ♂♂ + 4 ♀♀); 7. Ropočevo (S and M, 195 m, DQ63, 13 ♂♂ + 9 ♀♀); 8. Koceljeva (S and M, 128 m, DQ02, 22 ♂♂); 9. Jagodnja (S and M, 611 m, CQ60, 10 ♂♂ + 3 ♀♀); 10. Trstenik (S and M, 250 m, EP02, 9 ♂♂).

Western *variegata* group 11. Moluhe (B and H, 230 m, CQ13, 23 ♂♂ + 12 ♀♀); 12. Tešanj (B and H, 320 m, YK34, 10 ♂♂ + 8 ♀♀); 13. Vranica (B and H, 1690 m, YJ27, 15 ♂♂ + 5 ♀♀); 14. Zvijezda (B and H, 990 m, CP09, 6 ♂♂ + 9 ♀♀); 15. Pediše (B and H, 860 m, CP16, 5 ♂♂ + 5 ♀♀).

Northern *scabra* group 16. Tara (S and M, 1200 m, CP66, 21 ♂♂ + 14 ♀♀); 17. Vasiljev vrh (S and M, 1519 m, DP20, 20 ♂♂ + 10 ♀♀); 18. Banjica (S and M, 322 m, EN99, 17 ♂♂ + 6 ♀♀); 19. Dojkinci (S and M, 831 m, FN48, 14 ♂♂ + 5 ♀♀); 20. Zvonce (S and M, 661 m, FN25, 16 ♂♂ + 6 ♀♀); 21. Kukavica (S and M, 1287 m, EN73, 6 ♂♂ + 9 ♀♀); 22. Bosilegrad (S and M, 700 m, FN20, 15 ♂♂ + 4 ♀♀); 23. Prohor Pčinjski (S and M, 436 m, EM78, 15 ♂♂ + 10 ♀♀); 24. Erenik (S and M, 370 m, DM48, 12 ♂♂ + 5 ♀♀); 25. Biogradsko jezero (S and M, 1099 m, CN85, 14 ♂♂ + 3 ♀♀); 26. Bukumirsko jezero (S and M, 1440 m, CN81, 5 ♂♂ + 11 ♀♀); 27. Prekornica (S and M, 1380 m, CN52, 11 ♂♂ + 20 ♀♀); 28. Zagarač (S and M, 670 m, CN31, 10 ♂♂ + 8 ♀♀); 29. Bjeloši (S and M, 878 m, CM29, 18 ♂♂ + 10 ♀♀); 30. Livari (S and M, 515 m, CM56, 8 ♂♂ + 11 ♀♀).

Southern *scabra* group 31. Podgorci (FYROM, 910 m, DL66, 11 ♂♂ + 8 ♀♀).

Morphometric character descriptions

L, total length from the top of the snout to the edge of the cloaca; F, distance from the center of the cloaca to the knee joint; T, distance from the knee joint to the tibio-tarsal ankle; N, distance from the tibio-tarsal ankle to the top of the longest toe; P, distance from the metatarsal ankle to the top of the longest toe; H, distance from the shoulder joint to the elbow joint; M, distance from the elbow joint to the top of the longest finger; DpPa, length of the thumb on the front leg; DsPa, length of the 2nd finger on the front leg; DpPp, distance from the metatarsal tubercle to the top of the thumb; Cint, largest distance of the metatarsal tubercle; Lc, head length from the top of the snout to the corner of the mouth; Ltc, head width at the angle of the jaw; Spp, shortest distance between the inner eyelids; Spi, distance between the nostrils; Spcr, distance between the front end of the eyes; Lo, largest length of the eyes; Ltp, largest width of the upper eyelid; Dro, distance between the top of the snout and the front end of the eye; and Dno, distance between the nostrils and the front end of the eye.