A puzzle about *Bombina* sp.: a yellow-bellied specimen of the fire-bellied toad (*Bombina bombina* Linnaeus, 1761) indicates the highest proven habitat of the species in Bulgaria

Nikolay Natchev^{1,2,*}, Daniel Jablonski³, Georgy Dashev¹, Teodora Koynova¹, Dimcho Zahariev¹ and Nikolay Tzankov⁴

Abstract. To date, the data published on the occurrence of the fire-bellied toad (*Bombina bombina*) and yellow-bellied toad (*B. variegata*) in the region of the Natural Park "Shumensko plato" (SE Bulgaria) are controversial. The species are included in the inventory of the protected territory, but none of them has been reported since 1934. In the range of this study we provide a field survey to investigate whether both *Bombina* species inhabit the park and where they can be located. We selected two study polygons where water basins are available for the toads at least in the spring. During our four year investigation we were able to detect only a single fire-bellied toad. One female specimen was captured in the spring of 2012 and recaptured in 2014. The specimen was found at an altitude of 472 m a.s.l. This is the highest habitat reported for *B. bombina* in Bulgaria.

Key words. amphibian, anuran, field survey, monitoring, elevation, zoogeography

Introduction

The fire-bellied toad (*Bombina bombina* Linnaeus, 1761) inhabits Central and Eastern Europe (Sillero et al., 2014) where it is usually associated with lowlands. However, there is no general geographic pattern in the altitudinal distribution of the species. It has been reported at 732 m a.s.l. in the Czech Republic (Zavadil, 1993); at 650 m a.s.l. in Slovakia (Lác, 1968); at 590 m a.s.l. in Austria (Cabela et al., 2001); up to approximately 420 m a.s.l in Romania (Strugariu et al., 2008; Gherghel et al., 2008, but see the altitudinal range in Rozylowicz et

- ² Department of Integrative Zoology, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria
- ³ Department of Zoology, Comenius University in Bratislava, Mlynská dolina B-1, 842 15, Bratislava, Slovakia
- ⁴ Section Vertebrates, National Museum of Natural History, Bulgarian Academy of Sciences, Tsar Osvoboditel 1, 1000 Sofia, Bulgaria
- * Corresponding author e-mail: nikolay.natchev@univie.ac.at

al., 2013). The highest altitude record for this species was reported from Russia (830 m a.s.l.) on the northern foothills of Caucasus (Kuzmin, 2012). In some areas the maximum altitude of *B. bombina* populations is lower: up to 250 m a.s.l. in Poland (Szymura, 2003), 350 m a.s.l. in Ukraine (Ščerbak and Ščerban, 1980) or up to 300 m a.s.l in Germany (Günther and Schneeweiss, 1996).

Since the end of the 19th century, the general pattern of the species distribution in Bulgaria has been well studied and documented through the years (e.g. Kovachev, 1894, 1905, 1912; Buresch and Tsonkov, 1942; Angelov and Kalchev, 1961; Beškov and Beron, 1964; Beškov, 1972; Undjian, 2000; Beshkov and Nanev, 2002; Stojanov et al., 2011). In Bulgaria, B. bombina occurs in the Danubian and the Thracian lowlands and also sporadically along the Black sea coast (Stojanov et al., 2011). The species prefers natural and artificial lakes, temporary puddles or the vicinity of large running rivers and channels (Stojanov et al., 2011). According to these authors, B. bombina occurs from the sea level commonly up to an elevation of 250 m in Bulgaria. To date, the highest published record (450 m a.s.l.) is from the area "Devetashko plato" (Tzankov and Popgeorgiev, 2014).

¹ Faculty of Natural Sciences, Shumen University, Universitetska str. 115, 9700 Shumen, Bulgaria



Figure 1. Geographic position of localities mentioned in the text: 1 represents "Shumensko plato" (presented here); 2 represents Svalenik village [data from Unjian (2000)]; 3 represents the mouth of Kamchia river (presented here).

The Natural Park "Shumensko plato" is positioned on a plateau which is higher than the "Devetashko plato" and a potential record of the fire-bellied toad would indicate the highest habitat of the species on Bulgarian territory. The existing records on the presence of *B. bombina* and *B. variegata* in the region are very puzzling. The earliest descriptions are rather incomplete and inaccurate (see Kovachev, 1905; Buresch and Tsonkov, 1942). Neither of the two species has been confirmed in the area afterwards. To revise the presence of *B. bombina* and *B. variegata* in the territory of the Natural Park "Shumensko plato", in the range of the present study we conducted field investigations in the region of the plateau.

Material and methods

We conducted surveys in the spring of the years 2011 to 2014. Investigation of the water bodies was carried out in two defined areas: "Kioshkovete place" and the Memorial "1300 Years Bulgaria" (respectively southwest and south from the center of the Shumen town, NE Bulgaria). Both localities were selected because the "Kioshkovete place" is positioned at the foothills of "Shumensko plato" and the Memorial is on the top of the plateau (denivelation of about 150 m). In both areas water basins were available (at least in spring time). The monitoring took place from March to July at irregular intervals from 28 to 45 days.

During our herpetological research, a single specimen of *B. bombina* was captured in 2012 and recaptured in 2014. It was identified as fire-bellied toad based on the following characters (sensu Stojanov et al., 2011): general pattern composition and arrangement of the ventral coloration with predominant dark base color instead of orange color patches; great fragmentation of the patches; head ratio (the length was greater than the width); dorsal tubercles rounded and smoothed, white spots around the dorsal tubercles.

Results and discussion

On 18 April 2012 in a temporary puddle on the forest road near the Memorial "1300 Years Bulgaria", southwest from Shumen (43.246°N 26.911°E, 471 m a.s.l; see Figure 1) we documented an adult *B. bombina* female and its eggs. On 26 April 2014 the same individual was recaptured in a water body (approximately 15x15



Figure 2. Ventral coloration of recorded *Bombina bombina* from Natural Park "Shumensko plato": A, individual found in 2012; B, same individual recaptured in 2014).

m) at about 500 m southwest (43.247°N 26.905°E) from the spot of the first finding in 2012. For the second locality, based on GPS coordinates verified on a 1:5000 georeferenced topographic map, the altitude was 472 m a.s.l. This is the highest officially recorded elevation of a *B. bombina* habitat for Bulgaria. The ventral coloration of the specimen was orange in 2012 and pale yellow in 2014 (Figure 2). The documented specimen of *B. bombina* is the only toad of the genus *Bombina* ever recorded on the top of the plateau. Thus, we will discuss this finding with respect to several important factors.

Habitat specifications of the region of the Memorial "1300 Years Bulgaria" in the Natural Park "Shumensko plato" and recent occurrence of the species in NE Bulgaria

Both localities where the fire-bellied toad was found can be classified as the following habitat types: habitat type 41.2B Pannonic oak-hornbeam forests (according to Palearctic habitat classification of Moss and Davies, 2002); G1.A1 [*Quercus*] – [*Fraxinus*] – [*Carpinus betulus*] woodland on eutrophic and mesotrophic soils; G1.A1C Southeastern European oak-hornbeam forests; G1.A1C3 Moesian oak-hornbeam forests (according to EUNIS habitat classification) - The habitat is listed in Supplement I of the habitat directive 92/43 and respectively Supplement I of the Bulgarian Biodiversity Law as "91G0* Pannonic woods with *Quercus petraea* and *Carpinus betulus*". The categories are included in the management plan of Natural Park "Shumensko plato" from 2011. According to all classifications, the habitat is of high priority. It is classified as endangered within the European Union and is an object of strong protection (European Directive 92/43/EEC).

According to the hypothesis on the postglacial recolonization routes of *Bombina* species presented in Arntzen (1978), dispersal routes of *B. bombina* followed the main river floodplains including the region of the Danube valley. The population of fire-bellied toads in the region near the town of Shumen could be a remnant of a historical recolonization from close refuges located along the Black Sea coast and the Danube River (see Fijarzcyk et al., 2011). The dispersion and penetration towards smaller river valleys seem to have been greatly facilitated by human activity; e.g. Vines et al. (2003) proposed that the colonization of the Transylvanian Plateau happened about 10 Kya.

The microhabitat in "Shumensko plato" (Figure 3) is not typical for *B. bombina* (see Stojanov et al., 2011). In Bulgaria, the fire-bellied toad is associated with lowlands and permanent and predominantly stagnant water basins, mostly in open habitats. The present distribution of *B. bombina* in Bulgaria was summarized by Stojanov et al. (2011). However, the map presented there does not provide exact localities. The locality described in the present study is isolated from the core distribution area of this species. The closest known exact records of *B. bombina* are from Rusenski Lom valley near Svalenik



Figure 3. Photos of microhabitats where the female firebellied toad was captured: A, in 2012; B, in 2014.

village (Unjian, 2000) and at the mouth of Kamchia River (N. Tzankov, unpublished data). Both are over 70 km away from "Shumensko plato" (see Figure 1).

Historical impact on current situation of *Bombina* spp. in Shumensko plato

It was Kovachev (1905) who first mentioned "locality Shumen" for *B. bombina*, but without any further specifications. Buresch and Tsonkov (1942) reported the presence of *B. variegata* in puddles at "Kioshkovete" place. On 16th July 1934, I. Tsonkov euthanized two specimens of toads for the collection of the Bulgarian Royal museum, but these were not found in the collection of the National Museum of Natural History in Sofia. Apparently the specimens had been lost. Currently there is no reliable information whether the yellow-bellied toad is part of the batrachofauna of the Natural Park "Shumensko plato" and whether the two *Bombina* species hybridize as reported for other sympatric populations of toads (see Szymura, 1993).

Hybridization between *Bombina* species is well documented. Different species hybridize in contact

areas in a large scale all along their distributional ranges (Szymura, 1993). Clinal, mosaic or both types of hybrid zones were reported for, e.g. Austria (Gollmann, 1984), Poland (Szymura and Barton, 1986), Hungary and Slovakia (Gollmann et al., 1988), Croatia (MacCallum et al., 1998), Romania (Vines et al., 2003) and Ukraine (Yanchukov et al., 2006). Differences in habitat preferences have a crucial role in hybrid zones (see MacCallum et al., 1998; Vines et al., 2003; Yanchukov et al., 2006). Extensive multidisciplinary studies (Gollmann, 1984, 1987; Gollmann et al., 1988; Szymura and Barton, 1991; Nürnberger et al., 2005; Szymura, 1983, 1993, 1995; Kruuk et al., 1999; Szymura et al., 2000; Vines et al., 2003) demonstrated that for the determination of hybrid individuals, morphological traits (color pattern, morphometrics etc.) are not a reliable indicator. For example, the quantification of geometric and color-defined characters of ventral patches proposed by Vörös et al. (2007) discriminated the parent species well enough, but could not be fully applied to hybrids.

Co-occurrence of the fire-bellied and the yellowbellied toad in Bulgaria is rare. It was first mentioned by Beškov et al. (1967) and recently confirmed (N. Tzankov and M. Slavchev, unpublished data). Only one fertile hybrid population in Chernelka gorge was reported for Bulgaria (see Jameson et al., 1982). It is possible that the identification of the specimens found by I. Tzonkov in 1934 in "Kioshkovete" place as *B. variegata* was erroneous and he actually had found yellow-colored *B. bombina* specimens. The presently reported ontogenetic shift in the ventral coloration will be in the scope of future studies on the local population.

Photographic documentation as an important marking method for identifying *Bombina* individuals

There are different invasive marking methods for amphibians. The most important and frequently used are toe clipping and cutaneous coloration (for overview see Bayley and Nichols, 2009; Ferner, 2009). Most of the methods are very effective for short time monitoring (up to one season). Long time monitoring requires expensive marking with subcutaneous chips or the use of photo documentation. Thus, when estimating the population size of *B. bombina*, often individual identification is based on unique belly pattern features or on pattern mapping (Sas et al., 2005). The color features change slightly during ontogenetic development (see Figure 2), but nevertheless are a reliable indicator for the identification of every single specimen. Different widely used schemes for *Bombina* specific determination were adapted for populations from the northern part of the distribution area of the species. The pattern scheme presented in Michalowski (1958) and later adopted by Lác (1961) and Michalowski and Madej (1969) is based on the percentage of patch coverage. The system of Stugren (1959) is based on patch connectivity and is used mostly by Romanian herpetologists [for example recent work by Sas et al. (2005), Covaciu-Marcov et al. (2009) etc.]. Gollmann (1984) and Szymura & Barton (1991) proposed combined, optimized schemes.

In conclusion we have to point out that many of the traits listed in the proposed identification algorithms are not useful for the mixed East Balkan populations. The recognition schemes have to be adapted for the local populations and a databank of photos of the ventral coloration in *B. bombina* and *B. variegata* has to be established. This will allow scientists to identify individuals, to provide comparative morphological studies and to fine-tune the individual recognition system.

Acknowledgments. We thank the administration of the Shumen University for supporting a sophisticated, modern and interactive educational program. The authors would like to acknowledge M. Rindoš and also the members of the workgroup "Ethnobotanics" at the Shumen University for their support during the field surveys. Dr. Georgy Popgeorgiev provided a GIS database and designed the maps we used.

References

- Angelov, P., Kalchev, B. (1961): The amphibians in the Natural history museum in Plovdiv. Nature and Knowledge 14 (2): 18-21 (in Bulgarian).
- Arntzen, J. W. (1978): Some hypotheses on postglacial migration of the Fire-bellied toad, *Bombina bombina* and the Yellowbellied toad, *Bombina variegata*. Journal of Biogeography 5: 339-345.
- Bailey, L.L., Nichols, J.D. (2009): Capture–mark–recapture, removal sampling, and occupancy models. In: Amphibian Ecology and Conservation. A handbook of techniques, p. 447-465. Dott, K., Ed., Oxford University press.
- Beshkov, V., Nanev, K. (2002): Amphibians and reptiles in Bulgaria. Sofia-Moscow, Pensoft.
- Beshkov, V., Undshian, E., Simeonov, S., Daraktschiev, A. (1967): Neue Angaben über die Verbreitung einiger Amphibien und Reptilien in Bulgarien. – Bulletin de l'Institut de Zoologie et Musée, Sofia 25: 5-10.
- Beshkov, V. (1972): Zwischenartenkontakte und Zusammenleben der Frösche in Bulgarien. – Bulletin de l'Institut de Zoologie et Musée, Sofia 34: 85-95.

- Beshkov, V., Beron, P. (1964): Catalogue et bibliographie des Amphibiens et des Reptilies en Bulgarie. Sofia, ABS.
- Buresch, I., Tsonkov, J. (1942): Tailless amphibians (Amphibia, Salientia). Proceedings of the royal natural institute. Sofia 15: 68-165.
- Cabela, A., Grillitsch, H., Tiedemann, F. (2001): Atlas zur Verbreitung und Ökologie der Amphibien und Reptilien in Österreich: Auswertung der Herpetofaunistischen Datenbank der Herptologischen Sammlung des Naturhistorischen Museums in Wien. Umweltbundesamt, Wien.
- Covaciu-Marcov, S.D., Fereni, S., Bogdan, H.V., Groza, M., Bata, Z.Ş. (2009): On the hybrid zone between *Bombina bombina* and *Bombina variegata* in Livada Forest, north-western Romania. Biharean Biologist 3: 5-12.
- D.G. Environment. (1992): Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. Directorate-General Environment, European Commission. – Official Journal, N. L. 206: 22/07/1992.
- Ferner, J.W. (2009): Measuring and marking post-metamorphic amphibians. In: Amphibian Ecology and Conservation. A handbook of techniques, p. 121-143. Dott, K., Ed., Oxford University press.
- Fijarczyk, A., Nadachowska, K., Hofman, S., Litvinchuk, S.N., Babik, W., Stuglik, M., Gollmann, G., Choleva, L., Cogalniceanu, D., Vukova, T., Dzukic, G., Szymura, J.M. (2011): Nuclear and mitochondrial phylogeography of the European fire-bellied toads *Bombina bombina* and *Bombina variegata* supports their independent histories. Molecular ecology **20** (16): 3381-3398.
- Gherghel, I., Strugariu, A., Ghiurcă, D., Cicort-Lucaciu, A.Şt. (2008): The herpetofauna from the Bistriţa river basin (Romania): geographical distribution. North Western Journal of Zoology 4: 71-103.
- Gollmann, G. (1984): Allozymic and morphological variation in the hybrid zone between *Bombina bombina* and *Bombina variegata* (Anura, Discoglossidae) in north-eastern Austria. Zeitschrift für Zoologische Systematik und Evolutionsforschung 22: 51–64.
- Gollmann, G. (1987): Bombina bombina and Bombina variegata in the Mátra mountains: new data on distribution and hybridisation. Amphibia–Reptilia 8: 213–224.
- Gollmann, G., Roth, P., Hödl, W. (1988): Hybridisation between the fire-bellied toads *Bombina bombina* and *Bombina variegata* in the karst regions of Slovakia and Hungary: morphological and allozyme evidence. Journal of Evolutionary Biology 1: 3–14.
- Günther, R.N., Schneeweiss, N. (1996): Rotbauchunke Bombina bombina (Kinnaeus, 1761). In: R. Günther (ed.), Die Amphibien und Reptilien Deutschlands. Jena, Stuttgart, Lübeck, Ulm, Gustav Fischer Verlag..
- Jameson, D., Smith, M., Dove, L., Klass, M., Beshkov V. (1982): Analysis of a hybrid population of *Bombina (bombina X variegata)*. 62 annual meeting, american society of ichthyologists and herpetologists, N. Illinois University, Illinois, DeKalb.
- Kovachev, V. (1894): Materials for studying the Bulgarian fauna – Periodical journal of the Bulgarian Literary Society 47: 746-747 (in Bulgarian).

- Kovachev, V. (1905): Contribution for the studying of the amphibians and reptiles in Bulgaria. Book of folklore. 21: 1-13 (in Bulgarian).
- Kuzmin, S.L. (2012): Amphibians of the ex-USSR. Moscow, Creativity of scientific editions KMK
- Lác, J. (1961): Rozšírenie kuncov (*Bombina bombina* L. a *Bombina variegata* L.) na Slovensku a k problematike ich vzájomného kríženia. Biologické práce **59**: 5-32.
- Lác, J. (1968): Obojživelníky Amphibia. In: Stavovce Slovenska I – Ryby, obojživelníky a plazy, p. 231-312. Oliva, O., Hrabě, S., Lác, J., Eds., SAV, Bratislava.
- MacCallum, C.J., Nürnberger, B., Barton, N.H., Szymura J.M. (1998): Habitat preference in a *Bombina* hybrid zone in Croatia. Evolution **52**: 227–239.
- Michałowski, J. (1958): Geographische Verbreitung der Unken (Bombina Oken) zwischen den Flüssen Weichsel, Skawa und Raba (Woiwodschaft Krakow). Acta Zoologica Cracoviensia 3: 247-283.
- Michałowski, J., Madej, Z. (1969). Studies on the relationship of *Bombina bombina* (Linnaeus) and *Bombina variegata* (Linnaeus). III. Taxonomic characters from laboratory and in interspecific hybrids. - Acta Zoologica Cracoviensia 14: 173-202.
- Moss, D., Davies, C. (2002): Cross-references between the EUNIS habitat classification and the Palaearctic habitat classification, Centre for Ecology and Hydrology, CEH Monks Wood, Abbots Ripton. UK, Huntingdon, Cambs.
- Nürnberger, B., Barton N.H., Kruuk L.E.B., Vines T.H. (2005): Mating patterns in a *Bombina* hybrid zone: inferences from adult and full sib genotypes. Heredity 94:247-257.
- Rozylowicz, L., Cogălniceanu, D., Székely, P., Samoilă, C., Ruben, I., Tudor, M., Plăiaşu, R., Stănescu, F. (2013). Diversity and distribution of amphibians in Romania. ZooKeys 296: 35-57.
- Sas, I., Covaciu-Marcov, S.D., Pop, M., Ile, R. D., Duma, C. (2005): About a closed hybrid population between *Bombina bombina* and *Bombina variegata* from Oradea (Bihor county, Romania). North-Western Journal of Zoology 1: 41-60.
- Ščerbak, N.N., Ščerban, M.I. (1980): Amphibians and Reptiles of Ukranian Charpatians. Kiev, Naukova Dumka.
- Sillero, N., Campos J., Bonardi A., Corti, C., Creemers, R., Crochet, P.A., Crnobrnja-Isailović, J., Denoël, M., Ficetola G.F., Gonçalves, J., Kuzmin, S., Lymberakis, P., de Pous, P., Rodríguez, A., Sindaco, R., Speybroeck, J., Toxopeus, B., Vieites, D.R., Vences, M. (2014): Updated distribution and biogeography of amphibians and reptiles of Europe. Amphibia-Reptilia, **35**: 1-31.
- Stojanov, A., Tzankov, N., Naumov, B. (2011) Die Amphibien und Reptilien Bulgariens Frankfurt am Main, Chimaira.
- Strugariu, A., I., Gherghel, S.R., Zamfirescu, T., Sahlean, C. (2008): Spatial distribution of the herpetofauna from the upper and middle Moldova river basin (Romania). Travaux du Museum National d'Histoire Naturelle "Grigore Antipa" 51: 231-241.

- Stugren, B. (1959): Eidonomische Untersuchungen an *Bombina* Oken (Amph., Discoglossidae) aus dem Gurghiu-Tale (Siebenbürgen). Zoologische Jahrbücher, Abteilung Systematik 86: 382-394.
- Szymura, J.M. (1983): Genetic differentiation between hybridizing species *Bombina bombina* and *Bombina variegata* (Salientia, Discoglossidae) in Poland. Amphibia–Reptilia 4: 137–145.
- Szymura, J.M. (1993): Analysis of hybrid zones with *Bombina*. In: Hybrid zones and the evolutionary process, p. 261-289. Harrison, R.G., Ed., Oxford, Oxford University Press.
- Szymura, J.M. (1995): Inheritance of allozyme loci in *Bombina*: one linkage group established. Biochemical Genetics **33**: 167– 172.
- Szymura, J.M. (2003): Kumak nizinny Bombina bombina (Linnaeus, 1761). In: Atlas płazów i gadów Polski. Status-Rozmieszczenie-Ochrona, p. 1-125. Rafiński, J., Ed., Warszawa-Kraków, Biblioteka Monitoringu Środowiska.
- Szymura, J.M., Barton, N.H. (1986): Genetic analysis of a hybrid zone between the fire-bellied toads, *Bombina bombina* and *Bombina variegata*, near Cracow in Southern Poland. Evolution 40: 1141-1159.
- Szymura, J.M., Barton, N.H. (1991): The genetic structure of the hybrid zone between the fire-bellied toads *Bombina bombina* and *B. variegata*: comparisons between transects and between loci. Evolution 45: 237–261.
- Szymura, J. M., Uzzell, T., Spolsky. C. (2000): Mitochondrial DNA variation in hybridizing fire-bellied toads, *Bombina bombina* and *B. variegata*. Molecular Ecology **9**: 891–899.
- Tzankov, N., G. Popgeorgiev. (2014): Conservation and declines of Amphibians in Bulgaria. In: Amphibian Biology Volume 11,: Status of Conservation and Decline of Amphibians: Eastern Hemisphere Part 4: Southern Europe and Turkey, p. 131-139. Heatwole H., Wilkinson J.W., Eds., Pelagic Publishing.
- Unjian, E. (2000): Investigation of the vertebrates on the valley of Lomovete and the territory of Natural Park "Rusenki Lom" – districts Ruse and Razgrad. III. Amphibians, IV. Reptiles. Pub. Natural Park "Rusenki Lom".
- Vines, T.H., Köhler, S.C., Thiel, M., Ghira, I., Sands, T.R., MacCallum, C.J., Barton, N.H., Nürnberger, B. (2003): The maintenance of reproductive isolation in a mosaic hybrid zone between the fire-bellied toads *Bombina bombina* and *B. variegata*. Evolution **75**: 1876-1888.
- Yanchukov, A., Hofman, S., Szymura, J.M., Mezhzherin, S.V., Morozov-Leonov, S.Y., Baton, N.H., Nürnberger, B. (2006): Hybridization of *Bombina bombina* and *B. variegata* (Anura, Discoglossidae) at a sharp ecotone in Western Ukraine: comparisons across transects and over time. Evolution **60**: 583-600.
- Zavadil, V. (1993): Vertikale Verbreitung der Amphibien in der Tschechoslowakei. Salamandra 28: 202-222.