

Herpetofauna Bulgarica – nomina actuales. Amphibia

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Abstract: Updated systematic and taxonomic status of Bulgarian amphibians currently comprises 23 nominal species (24 taxa). Eight tailed and 15 tailless amphibian species are listed. For each taxa, important clarification and explanation notes are presented. As well, future challenges and prospects are denoted. There are still many phylogeographic questions to be resolved and contacts between closely related taxa to be studied. More notably for two groups at present a precise systematic definition could not be given, e.g. *Bufo viridis* complex and *Pelophylax ridibundus* complex.

Key words: amphibians, Bulgaria, checklist, systematic, taxonomy

Introduction

After the last work dealing with systematic and taxonomy of Bulgarian amphibians and reptiles (TZANKOV, 2011), a substantial amount of papers appeared which raises the necessity of actualization of the national checklist. Some changes were briefly announced in TZANKOV & POPGEORGIEV (2014). The taxonomy of higher ranks is in accordance to that proposed by FROST (2016) except for the class Amphibia where the authorship used by BLACKBURN & WAKE (2011) is followed. The current list implements the latest researches concerning and affecting the systematic and taxonomy of Bulgarian amphibians. It is adopted to be in use for various researches that communicate with systematic and taxonomic of these taxa.

Systematic and taxonomic account

Class Amphibia Gray, 1825

Order Caudata Fischer von Waldheim, 1813

Family Salamandridae Goldfuss, 1820

Subfamily Pleurodclinae Tschudi, 1838

Genus *Ichthyosaura* Sonnini de Manoncourt et Latreille, 1801

Species *Ichthyosaura alpestris* (Laurenti, 1768)

Subspecies *Ichthyosaura alpestris reiseri* (Werner, 1902)

Genus *Lissotriton* Bell, 1839

Species *Lissotriton graecus* (Wolterstorff, 1906)²

Species *Lissotriton vulgaris* (Linnaeus, 1758)

Subspecies *Lissotriton vulgaris schmidtleri* (Raxworthy, 1988)²

Subspecies *Lissotriton vulgaris vulgaris* (Linnaeus, 1758)

Species *Triturus cristatus* (Laurenti, 1768)

Species *Triturus dobrogicus* (Kiritzescu, 1903)⁴

Species *Triturus ivanbureschi* Arntzen et Wielstra, 2013^{4,5}

Species *Triturus macedonicus* (Karaman, 1922)⁶

Subfamily Salamandrinae Goldfuss, 1820

Genus *Salamandra* Garsault, 1764

Species *Salamandra salamandra* (Linnaeus, 1758)⁷

Order Anura Fischer von Waldheim, 1813

Family Bombinatoridae Gray, 1825

Genus *Bombina* Oken, 1816

Species *Bombina bombina* (Linnaeus, 1761)⁸

Species *Bombina variegata* (Linnaeus, 1758)⁹

Family Pelobatidae Bonaparte, 1850

Genus *Pelobates* Wagler, 1830

Species *Pelobates fuscus* (Laurenti, 1768)¹⁰

Species *Pelobates syriacus* Boettger, 1889

Subspecies *Pelobates syriacus balcanicus* Karaman, 1928¹¹

Family Bufonidae Gray, 1825

Genus *Bufo* Garsault, 1764
 Species *Bufo bufo* (Linnaeus, 1758)¹²
 Genus *Bufo* Rafinesque, 1814
 Species *Bufo viridis* complex¹³
 Family Hylidae Rafinesque, 1815
 Subfamily Hylinae Rafinesque, 1815
 Genus *Hyla* Laurenti, 1768
 Species *Hyla arborea* (Linnaeus, 1758)¹⁴
 Species *Hyla orientalis* Bedriaga, 1890¹⁴
 Family Ranidae Batsch, 1796
 Genus *Pelophylax* Fitzinger, 1843
 Species *Pelophylax* kl. *esculentus* (Linnaeus, 1758)¹⁵
 Species *Pelophylax kurtmuelleri* (Gayda, 1940)¹⁶
 Species *Pelophylax lessonae* (Camerano, 1882)¹⁷
 Species *Pelophylax ridibundus* complex¹⁸
 Genus *Rana* Linnaeus, 1758
 Species *Rana dalmatina* Fitzinger, 1839
 Species *Rana graeca* Boulenger, 1891
 Species *Rana temporaria* Linnaeus, 1758

¹The combination *I. a. carpathica* was used in STOJANOV et al. (2011) pending on phylogenetic data provided by SOTIROPOULOS et al. (2007) and their future interpretation by DUBOIS & RAFFAELLI (2009). According to the latter phylogeographic analyses both South Carpathian and central Balkan subclades are supported thus the later should be attributed to the subspecies *I. a. reiseri* (Werner, 1902) (RECUERO et al., 2014). The very characteristic wide head of this subspecies pointed in the original description by WERNER (1902), could be interpreted as a local extreme variation. That is not the case for other neighboring populations (DUBOIS & RAFFAELLI, 2009). The taxonomic status of the so called “Vlasina lineage” have to be specify and finer scale studies are needed in order to clarify the gene flow processes across lineages (RECUERO et al., 2014).

² The phylogeographic study of PABIJAN et al. (2015) based on mitochondrial genes (ND2, ND4+tRNAs) revealed a very complex situation in the Balkan Peninsula. Their lineage D is an example for bidirectional exchange scenario and encompass both specimens morphologically attributed to *L. graecus* (at the west) or to *L. v. vulgaris* (at the east). Its presence in westernmost *L. graecus* population was interpreted as a recent introgression with the neighboring populations of the nominate subspecies. When regarding the situation with lineage K three scenarios were proposed. The first one suggest recent introgression from *L. v. vulgaris* into *L. graecus* with incomplete sorting of ancestral polymorphism. The second rely to recent acquisition during the range expansion or older introgression with recent demographic expansion of the introgressed *L. graecus*. The third propose an adaptive introgression into

L. v. vulgaris with K mtDNA by populations with genes underlying the *L. graecus* morphology. All the three scenarios could be tested with nuclear markers as suggested by PABIJAN et al. (2015).

PABIJAN et al. (2015) reported lineage E (found mostly in northwest Asia Minor) from two localities in South Central Bulgaria. This lineage fit with well with the subspecies *I. r. schmidlerorum*. DUBOIS & RAFFAELLI (2009) correctly pointed that it could be used under its original spelling *schmidleri* (RAXWORTHY, 1988).

Even if SPEYERBROECK et al. (2010) do not accept the specific status of *L. v. graecus* relying on biological species concept, its status could be well justified if relating to genus *Triturus*, where similar situation could be observed when for example the introgression of mitochondrial genome of *T. vanbureschi* into *T. macedonicus* in central Balkan Peninsula is taken into account (see WIELSTRA & ARNTZEN, 2012; ARNTZEN et al., 2014). Separate taxonomic status of ‘*graecus*’ and ‘*schmidleri*’ was also supported by combined analysis of allozymes, genome size and morphology (SKORIKOV, 2008, 2011).

³WIELSTRA et al. (2015) demonstrated the low intraspecific genetic variation in *T. cristatus* and refuted the existence of an entire glacial refugium south of the Danube River claiming on single mitochondrial haplotypes found there and on asymmetrically introgression by *T. ivanbureschi* mit. DNA. Obviously when analyzing the present day distribution, whether in terms of mit. DNA or nuclear introns (fig. 1 b,c in WIELSTRA et al. (2015) their clade 2 seems to have evolved in the region southwest of Carpathian Mt. and likely the Danube is not acting as a barrier. But when looking to the narrow introgression zone with *T. ivanbureschi* in Northwest Bulgaria the result could be interpreted in term of late (post-glacial) range expansion of the later in the region of North Bulgaria as the main dispersal route for this taxon seems to be south of the Balkan Mt. range. It is also unusual to interpret the spatial distribution models for the last glacial maximum events (fig. 5 in WIELSTRA et al., 2015) when the splits between and crowns of the three clades of *T. cristatus* indicated a quite older period – from 1,1 to 1,4 Mya.

⁴WIELSTRA et al. (2016) argued that *T. dobrogi-cus* should be considered as a monotypic species. Their study encompasses the whole species distributional range and demonstrates a genetic admixture and no geographic diversification. Thus the subspecies *T. d. macrostoma* (Boulenger, 1908) resurrected by LITVINCHUK & BORKIN (2000) should be synonymized with the nominate one.

⁵Balkan populations previously attributed to *T. karelinii*, *T. karelinii arntzeni* or *T. arntzeni* are described as a newly named species (WIELSTRA et al., 2013). This species also inhabit northwestern Asia Minor where exist a contact zone with *T. anatolicus* Wielstra et Arntzen, 2016 confined to northern Asia Minor.

Population described as belonging to *T. macedonicus* by NAUMOV & TZANKOV (2008) coming from locality Livade (Slavyanka Mt.) based on morphology data (Wolterstorff index and the shape of the vomerine teeth rows) was latter shown to belong to *T. ivanbureschi* by combined analyzes of mitochondrial and nuclear datasets (WIELSTRA & ARNTZEN, 2014). This species could persist along the western country border as in the neighboring countries some very closely located populations exists.

⁷Recently the time calibrated phylogeographic study (processing 10 mitochondrial and 13 nuclear genes) demonstrate that speciation processes in this species took place in Pleistocene (VENCES et al., 2014). Samples designated as *S. s. beschkovi* Obst, 1981 were included in earlier study done by STEINFARTZ et al. (2000) including single mit. gene (D-loop), failed to resolve the eastern populations group phylogeny (group C in their work). While allozyme data (single locus CK-1) revealed some differentiation processes in Southeastern Europe where a unique allele occurred in Southwestern Balkan Peninsula but with frequencies that vary and nowhere found alone across the sampled localities. This study questioned the status of *S. s. beschkovi* and future more detailed sampling should reveal and clarify its status.

⁸Intraspecific diversification was weakly supported by complete mitochondrial genome and nuclear genes (Ncx-1, Rag-1) phylogeny (FIJARCZYK et al., 2011; PABIJAN et al., 2013). Therefore, it is regarded as monotypic.

⁹PABIJAN et al. (2013) provided a highly resolved and time-calibrated phylogeny based on complete mitochondrial genomes suggesting Pleistocene diversification. FIJARCZYK et al. (2011) revealed the existence of separate Rhodopean I lineage supported by mitochondrial genes (Cyth) and partly by nuclear genes (Ncx-I, Rag-I) phylogeny. Thus, the status of the Balkan taxon *Bombina variegata scabra* Kuster, 1843 remains unclear.

¹⁰LITVINCHUK et al. (2013) synonymize *P. f. in-subricus* Cornalia, 1873 with *P. f. fuscus*. The eastern clade was treated as a subspecies *P. f. vespertinus* (Pallas, 1771) or as a distinct evolutionary species if pending on concept proposed by DE QUEIROS (2005).

¹¹VEITH et al. (2006) based on mit. genes (12S, 16S) provided phylogenetic data in support of this lineage but this study included only certain *P. syriacus* subspecies and failed to include samples from the nominate subspecies.

¹²Phylogeographic analyses done independently by GARCIA-PORTIS et al. (2012) and RECUERO et al. (2012) resurrect the taxonomy of the group. As a result. *Bufo spinosus* Daudin, 1803 was erected to a full species inhabiting Southwest Europe and Northwest Africa by the second team. The full species rank was future advocated by ARNTZEN et al. (2013). Thereby the taxon 'spinosus' should not be used for the rest of the distributional range. The taxon that occurs in the Balkan Peninsula, *B. bufo*, demonstrates subdivision into several subclades (see GARCIA-PORTIS et al., 2012) that have to be tested with nuclear markers.

¹³DUBOIS & BOUR (2010) demonstrate that generic name *Bufotes* Rafinesque, 1814 have a priority over *Pseudepidalea* Frost, Grant, Faivovich, Bain, Haas, Haddad, de Sá, Channing, Wilkinson, Donnellan, Raxworthy, Campbell, Blotto, Moler, Drewes, Nussbaum, Lynch, Green, & Wheeler, 2006. The initial purpose to split the taxon *B. viridis* to several taxa (STOCK et al., 2006) was based on molecular data analyses of two mit. genes (D-loop and ND+tRNAs). Later publication adding addition sequences of nuclear sequences (alpha- tropornyosine introns and RAG-1) generally support the initial subdivision but dataset for some taxa are missing (STOCK et al., 2008). Similar supporting results was obtained when Turkish populations was studied by mean of other mit. genes (16S rRNA. ÖZDEMİR & KUTRUB, 2007: D-Ioop and 12S rRNA. ÖZDEMİR et al., 2014). Samples from European Turkey (Tekirdag and Istanbul) were attributed to *B. variabilis* (Pallas, 1769) (ÖZDEMİR et al., 2014). The future persistence of this taxon to the north could be expected. Whereas the closest localities of *B. viridis* s.s. comes from Southwestern Ukraine (Cherson Oblast, Golija Pristan). Central Hungary (Orgovany) and Southern Greece (Peloponnes) (STOCK et al., 2006). Until extensive sampling throughout the Balkan Peninsula, both taxa can be expected and thus the combination *Bufotes viridis* complex is recommended.

¹⁴Both mostly parapatric taxa occurs in Bulgaria having a contact zone in the upper Struma valley area (see fig. 1 in DUFRESNES et al., 2015). Additionally a putative hybrid was reported from Vidin (GVOZDIK et al., 2015).

¹⁵FROST (2016) does not recognize as a separate taxonomic unit and mentioned it as a hybrid. For

this hybridogenetic species DUBOIS & GUNTHER (1982) introduced the evolutionary systematic category 'klepton' which is tend to be omitted in some recent papers (see HOFFMANN et al., 2015).

¹⁶Various works treated this taxon as a separate species. MARRACCI et al. (2011) provided cytogenetic data that evidently support the full species rank. Authors found differences in hybridization ladders and in chromosomes labeled in FISH analysis among this taxon and other closely related congeners. *P. ridibundus* and *P. cf. bedriagae* (as quoted by original meaning, see comment III for future interpretation of their results). PLÖTNER et al. (2012) based on mitochondrial genes (ND2, ND3) and nuclear introns (serum albumin I (SAI-I) and the non-long terminal repeat (non-L'LTR) retrotransposon RanaCR I. embedded into SAI-I) provided weakly support and do not fully resolve the phylogenetic position of *P. kurtmuelleri*. HOFMAN et al. (2015) based on complete mitochondrial genome better supported the separated status of *P. kurtmuelleri*.

¹⁷This taxon was already found and reported as "*Rana esculenta lessonae*" for Bulgaria but was synonymized with the nominate form (BESHKOV, 1965) until be briefly accounted and formally newly recognized in TZANKOV & POPGEORGIEV (2014).

¹⁸The taxon *P. ridibundus* have its type locality as originally quoted: "versus mare Caspium: Volgae et Iaiico" (PALLAS, 1771). It was latter restricted to Guryev (=Atyrau, Kazakhstan) by MERTENS & MÜLLER (1928). SCHNEIDER & EGIASARJAN (1991) by means of bioacoustics pointed about the closer resemblance between the mating calls from type territory and various localities in West Asia. The application of a set of molecular methods permitted to be determined two widely distributed subclades among others in West Palearctic (12S, ND3 mitochondrial genes, PLÖTNER & OHST, 2001: *cytb*, 16S mit. Genes, LYMBERAKIS et al., 2007; ND2, ND3 mit. genes, AKIN et al., 2010, PLÖTNER et al., 2010). Those inhabiting the type territory belongs to the eastern subclade as revealed by analyzing mit. genes (ND2, ND3) and nuclear introns (serum albumin intron 1 (SAI-1) and the embedded in it retrotransposon RanaCR 1) (PLÖTNER et al., 1011). This subclade is going to the west to South Russia (Penza province. Samara and Saratov regions. COI mit. gene. Ermakov et al., 2013.1014: Mari EI and Tatarstan republics. COI mit. gene and SAI-1. Svinin et al., 2015: ZAMALETDINOV et al., 2015). Additionally through Asia Minor. it was found in European Turkey and Northeastern Greece (BEIERLI et al., 1996: LYMBERAKIS et al., 2007: AKIN et al., 2010: PLÖTNER et al., 2010; HOTZ et al., 2013).

Its going westward to Keramoti (west of river Nestos delta) (HOTZ et al., 2013) and northward to Edirne (AKIN et al., 2010) and Igneada (HOTZ et al., 2013). The work of MARRACCI et al. (2011) also provided cytogenetic evidences for diversification of eastern and western subclades. It must be pointed that: *f. ridibundus*' samples in their work coming from Vranje (southern Serbia) could be referred to the western lineage and those from Igneada (European Turkey) and Orestias (Evros province, Northeastern Greece) respectively to the eastern lineage. It is apparently that the eastern subclade should be named *P. ridibundus*. Bioacoustic data from Southeast Bulgaria led to discriminate particular mating calls belonging to this clade coming from Eastern Rhodopi Mt. (river bank near v. Alamovtsi. 23.08.20 II. N 41.386° E 25.038°: river Varbitsa near v. Balabanovo, Druzhba neighbourhood. 13.07.2007. N 41.555° E 25.381°: small puddle near river bank. east from v. Chernichevo. 13.07.2007. 13.07.20 I O. N 41.347° E 25.800°: small dam near v. Kolets, 16.04.20 II. N 41.873° E 15.324°) westernmost Strandza Mt. (artificial pond north from v. Golyamo Krushevo. 28.06.2010. N 41.218° E 26.928°) and South Black Sea coast (Arkutino lake, continuously from 2007 to 1914. N 42.330° E 27.725°). Samples from Kazanlak and Kamchiya have predominantly characteristics of eastern subclade based on allozyme data (HOTZ et al., 1013).

The western subclade was reported from Alepu swamps. Kazanlak, Nessebar (based on mit. gene ND3. AKIN et al., 2010) plus Sozopol and Alepu swamps (12S rmit. gene. PLÖTNER & OHST, 2001). Thus a broad contact zone between both subclades seems to exist in Southeast Bulgaria.

DUBOIS & OHLER (1996 "1994") raised and stabilized the available taxon for the western clade – *Rana fortis* Boulenger, 1884. PLÖTNER (2005) also mentioned this name availability but unfortunately this does not provoke any future act depict its particular importance for the stabilization of the nomenclature. The combination *Pelophylax ridibundus* complex could be in use until future progress in this direction. Additionally the combination *f. bedriagae* is not applicable for Balkan populations. This taxon was described from Damas (r=Damascus, southern Syria) (CAMERANO, 1881 "1881") and fits with the well supported subclade confined to the Levant and Nile delta (AKIN et al., 2010, PLÖTNER et al., 20.10. 2012).

Comments and Conclusions

Currently the checklist of amphibians that occurs in Bulgaria comprises 23 nominal species (24 taxa).

Two groups pose unresolved taxonomic questions, *B. viridis* complex and *f. ridibundus* complex. They are of particular importance having wide distributional ranges and offering a suitable opportunity for various phylogeographic scenarios. That is true for many sister species/taxa having completely (*I. a. reiseri*/Vlasina lineage; *L. graecus*/*L. v. vulgaris*; *L. v. schmidleri*/*L. v. vulgaris*; *B. variegata* Rhodopean *I. B. variegata* neighboring lineage; *H. arborea*, *H. orientalis*) or partly (*T. cristatus*/*T. dobrogicus*, *T. cristatus*/*T. ivanbureschi*, *T. dobrogicus*/*T. ivanbureschi*) unexplored contact zones. Phylogeographic distribution and structuring of a set of taxa needs future study and clarification (*L. graecus*, *L. vulgaris*, *S. salamandra*, *B. bufo*, *P. fuscus*, *P. syriacus*, *P. kl. esculentus*, *P. lessonae*, *P. ridibundus*, *R. dalmatina*, *R. graeca*, and *R. temporaria*). The current national status of *T. macedonicus* deserves special attention as this taxon is with highest conservation priority (sensu Biological Diversity Act of Bulgaria and

Council Directive 92/43/EEC) and potentially very restricted distributional range.

Of particular interests are the interspecific relationships between the members of genus *Pelophylax*. In particular the interrelationships between the parent species *f. lessonae ridibundus* complex (western lineage) and their hybrid 1' k l. *esculentus* as the Danube valley denote their southern range distribution (except for *P. ridibundus* complex). The persistence of various diploid (LR) and triploid (LLR, LRR) hybrid forms should also be clarified along the Danube River. As well the contacts between *P. ridibundus* complex and *f. kurtmuelleri* needs future exploration. Instead of a greatly useful molecular approaches using various mitochondrial and mostly nuclear gene sequences the implementation and applications of an additional morphological and bioacoustical data is highly appreciate. They are still of great practical importance.

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Херпетофауна на България: актуални имена. Amphibia

Николай ЦАНКОВ

(Резюме)

Съставен е съвременен списък на земноводните в България. За 18 таксона подробно се описват таксономичните проблеми, които възникват при молекулярния и генетичния им анализи. Това е необходимо поради динамичното развитие на таксономията на земноводните с прилагането на съвременни методи.