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Taxonomic assessment and distribution of common toads (*Bufo bufo* and *B. verrucosissimus*) in Turkey based on morphological and molecular data

Nurhayat Özdemir^{1,*}, Cantekin Dursun¹, Nazan Üzümlü², Bilal Kutrup³, Serkan Gül¹

Abstract. The *Bufo bufo* species group includes four species distributed in the western Palearctic: *B. bufo*, *B. eichwaldii*, *B. spinosus* and *B. verrucosissimus*. Both *B. bufo* and *B. verrucosissimus* are known to occur in Turkey, but their range boundaries and the taxonomic status of *B. verrucosissimus* are still uncertain. In this study, we analyzed the variation in a set of morphological characters and in two mitochondrial and two nuclear DNA markers to address these questions. Phylogenetic analyses of sequence data support two main clades of common toads in Turkey, corresponding to *B. bufo* and *B. verrucosissimus*. The latter is subdivided into two allopatric subclades including populations along the Mediterranean and Black Sea coast, respectively. Discriminant analysis of morphological data showed separation among groups as defined by molecular analyses. We discuss these results and their implications for the evolutionary history of common toads in Turkey.

Keywords: amphibians, Anatolia, Caucasia, morphometry, mtDNA, phylogeography.

Introduction

The *Bufo bufo* species group consists of four species distributed in the western Palearctic: *B. bufo* (Linnaeus, 1758) from the larger part of Europe, *B. eichwaldii* Litvinchuk, Borkin, Skorinov and Rosanov, 2008 from the Talysh mountains of Azerbaijan and Iran, *B. spinosus* (Daudin, 1803) from North Africa, Iberia and much of France, and *B. verrucosissimus* (Pallas, 1814) from the Caucasus and Turkey (fig. 1). *B. spinosus* has traditionally been seen as a circum-Mediterranean subspecies (Mertens and Wermuth, 1960; Litvinchuk et al., 2008; Sinsch et al., 2009) but has been more recently shown to be restricted to the western Mediterranean (Recuero et al., 2012). For a summary of the taxonomic history of the *B. bufo* species group see Frost (2019).

Previous studies have documented the presence of both *B. bufo* and *B. verrucosissimus* in Turkey, but their range boundaries are not well delimited. Recuero et al. (2012) studied toad samples from four localities in Turkey (Samsun, Tokat, Karabük, and Artvin), and based on phylogenetic analyses of mitochondrial and nuclear genes, they assigned the population from Artvin to *B. verrucosissimus* and the others to *B. bufo*. In a study published in the same year, Garcia-Porta et al. (2012) also analyzed samples from Turkey. They assigned individuals from Alanya to their Caucasus clade (corresponding to *B. verrucosissimus*), and those from other localities in Turkey (Anayurt, Kayabaşı, Bursa, and Bafra) to their European clade (corresponding to *B. bufo*) based on mitochondrial DNA. In addition, they analyzed variation in allozymes in samples from Abant Lake that were correspondingly assigned to *B. verrucosissimus*.

In a subsequent study, Arntzen et al. (2013a) reconciled the results of the Garcia-Porta et al. (2012) and Recuero et al. (2012) papers, which arrived at contrasting taxonomic arrangements regarding the status of *B. spinosus* and *B. verrucosissimus*, considered at the subspecies level

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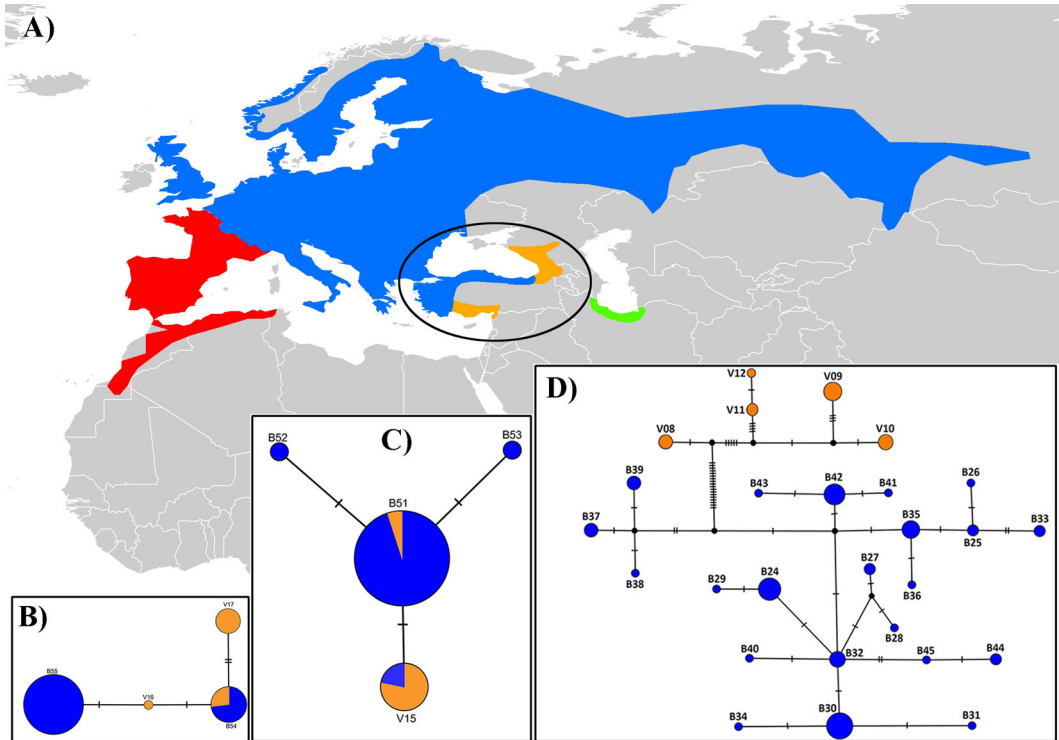


Figure 1. A distribution of common toad species in the western Palearctic, with *Bufo spinosus* in red (Arntzen et al., 2018), *B. bufo* in blue (Agasyan et al., 2009), *B. verrucosissimus* (this study) in orange, and *B. eichwaldi* (AmphibiaWeb., 2016) in green. Insets – haplotype networks of each nDNA gene (B: RPL3 gene; C: RAG1 gene) and the concatenated mtDNA sequences (D).

by Garcia-Porta et al. (2012) and as full species by Recuero et al. (2012). Arntzen et al. (2013a) reanalyzed the allozyme dataset of Garcia-Porta et al. (2012) and found no conclusive evidence for hybridization between *B. bufo* and *B. verrucosissimus*, including at the locality of Lake Abant, where individual-based genetic clustering analysis showed the simultaneous presence of the two species with no admixture. Accordingly, they called for maintaining species status for *B. verrucosissimus*.

In this study, we present new morphological and molecular data pertaining to the identification and spatial delineation of the Turkish *Bufo* species. Specifically, we aimed to provide and evaluate criteria for species identification and to further delineate species distributions of the two common toad species in Turkey.

Materials and methods

Sampling and morphometric analyses

A total of 193 adult *Bufo* toads (96 females, 97 males) were sampled during the spring breeding periods of 2015, 2016 and 2017 (supplementary table S1) in 25 Turkish localities (fig. 2). Prior to measuring, the toads were anesthetized with 250 mg/l MS222 and after processing they were released at the place of capture. The following 27 external morphological characters were measured following Castellano and Giacoma (1998), Orlova and Tuniyev (1989) and Arntzen et al. (2013b): snout vent length (SVL), length of the head (LHEAD), width of the head (WHEAD), minimum distance between the nostrils (INTNOS), distance between the nostril and the tip of snout (NOSTIP), minimum distance from the nostril to the anterior corner of the eye (NOSEYE), eye-tympanum distance (EYETYM), horizontal diameter of the eye (DEYE), diameter of the tympanum (DTYM), length of the parotoid (LPAR), distance between the elbows with humerus kept perpendicular to the body axis (WGRASP), radioulna length (RADUL), length of the hand (LHAND), length of the first finger (L1FING), length of the femur (LFEM), length of the tibia (LTIB), length of the tarsus (LTARS), length of the foot (LFOOT), minimum distance from the distal extremity of the inner metatarsal

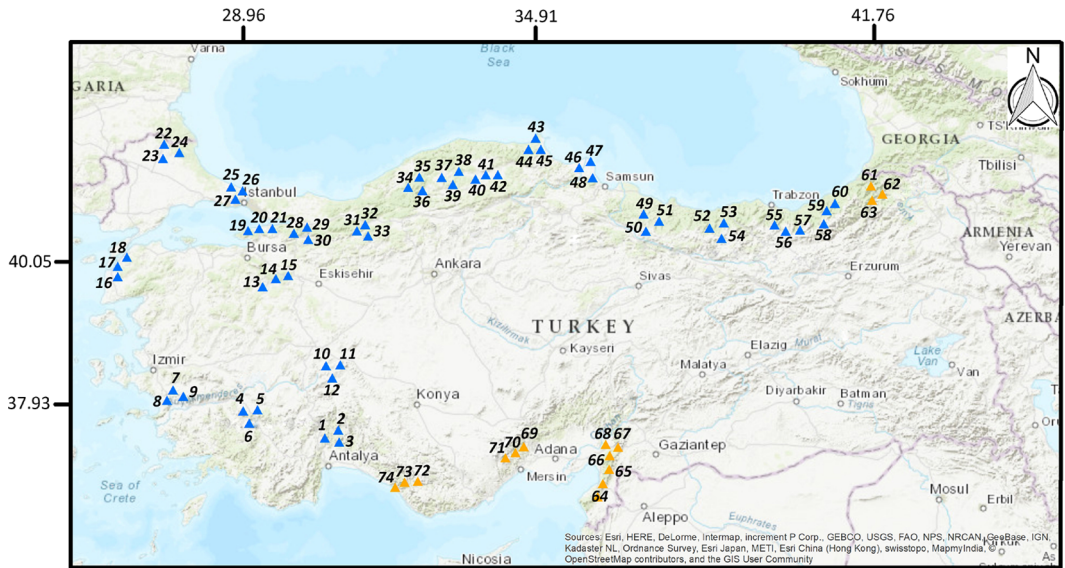


Figure 2. Detailed information on sampling locations in Turkey. Blue triangles represent *Bufo bufo* and orange triangles represent *Bufo verrucosissimus*. Locality numbers as in supplementary table S1.

tubercle and the web between the third and fourth digit (WEB), length of the metatarsal tubercle (LMET), interorbital distance (IOD), anterior parotoid distance (PDA), posterior parotoid distance (PDP), left parotoid width (LPW), right parotoid width (RPW), length of the inner metatarsal tubercle (LIMT) and width of the inner metatarsal tubercle (WIMT). Measurements were taken with digital calipers to the nearest 0.01 mm. To avoid inter-observer variation all measurements were done by the same researcher. We also studied the character parotoid angle (PA) and three derived characters, namely parotoid divergence ($PD = PDA/PDP$), MTSIZE (LIMT/SVL) and MTSHAPE (WIMT/LIMT) as referenced by Arntzen et al. (2013b). Finally, tissue samples (toe clips) were collected for molecular analyses. Samples were preserved in Eppendorf tubes containing 96% ethanol and kept in a freezer at -20°C until DNA extraction.

Molecular data and phylogenetic analyses

Genomic DNA was extracted from 74 ethanol-preserved tissue samples using the NucleoSpin Tissue Kit (Macherey-Nagel) following the manufacturer's instructions. Two mitochondrial (16S and cytochrome b) and two nuclear (RAG1 and RPL3) gene fragments were amplified by polymerase chain reaction (PCR) with the primer pairs 16Sar-16Sbr (16S, Palumbi et al., 1991), Amp-RAG1 F-Amp-RAG1 R1 (RAG1, San Mauro et al., 2004), and Cyt Bufo F-Cyt Bufo R (Cytb) and RPL3buF1-RPL3buR1 (RPL3, Recuero et al., 2012). PCRs were run in a T100 thermal cycler (BioRad) with 50 μl final volume, following protocols by Goebel et al. (1999) for 16S, Recuero et al. (2012) for Cytb and RPL3, and San Mauro et al. (2004) for RAG1. Amplified products were sequenced by Macrogen Europe Inc. (Amsterdam, The Netherlands).

We reconstructed haplotype networks using the median-joining algorithm for nuclear and mitochondrial gene fragments using PopArt 1.7 (Leigh and Bryant, 2015). Phylogenetic analyses were carried out on all DNA sequences listed in supplementary table S1, including outgroup sequences from the related toad species *B. gargarizans* Cantor, 1842, *B. bankorensis* Barbour, 1908 and *B. japonicus* Temminck and Schlegel, 1838. Sequences were aligned with ClustalW in BioEdit v7.2.5 (Hall, 1999). Inferred insertion/deletion polymorphisms (indels) were coded as informative characters with Fastgap v1.2 (Borchsenius, 2009). The best fitting nucleotide substitution model was selected with MrModelTest v2.3 for each gene separately, under the Akaike Information Criterion (Nylander, 2004). The SYM + G was the best-fit nucleotide evolution model for all genes and applied where appropriate. Phylogenetic trees were reconstructed with Bayesian (BI) and maximum likelihood methods (ML). We used MrBayes 3.2.6 (Ronquist and Huelsenbeck, 2003) for BI analyses. Four independent analyses were run for 10^6 generations (1.5×10^6 generations for concatenated data sets), after which the standard deviation of split frequencies was below 0.01 and the analysis was terminated. Each run included four chains (one cold and three heated), sampling one tree every 1000 generations. The first quarter of trees from each run was considered as burn-in and discarded. The remaining results were used to generate a 50% majority-rule consensus tree. The results were inspected with TRACER 1.7.1 (Rambaut et al., 2018) to check for convergence and for effective sample size values > 200 for each parameter in the model. ML analyses were performed with RaxML v8.0 (Stamatakis, 2014) with one thousand bootstrap replicates, under the GTRCAT model. Phylogenetic trees were visualized and edited with FigTree v 1.4.0 (Rambaut, 2012).

Lastly, we applied the species tree reconstruction method implemented in *BEAST under BEAST2 (Bouckaert et al.,

2019) to assess phylogenetic relationships in the *Bufo bufo* species group. Samples of *Bufo* species from Turkey were assigned to species based on their mtDNA. We also included published sequences of *B. spinosus*, *B. eichwaldi* and *Bufo gargarizans* as sequential outgroups. The concatenated dataset (mtDNA + nDNA) was considered as four independent partitions and the nuclear dataset as two independent partitions. Independent models of each partition were automatically selected using bModelTest (Bouckaert et al., 2017) integrated in BEAST2. Since all the mitochondrial genes are effectively linked due to a lack of recombination, 16S and Cytb genes were set up to use the same linked tree. The topologies and support values were built under the birth-death tree prior and relaxed lognormal clock. One independent analysis was run for 12×10^7 MCMC generations, sampling every 5000 steps. Convergence and ESS were checked using Tracer 1.7.1. (Rambaut et al., 2018) to confirm parameters were >200 . Afterwards, a Maximum Clade Credibility (MCC) tree was generated in TreeAnnotator with %20-burn-in and visualized using FigTree v.1.4.0 (Rambaut et al., 2012).

To strengthen the *BEAST results, Bayesian Factor Delimitation (BFD) analysis was further conducted with different species arrangements. BFD approach is allowing to compare models that contain different numbers of species especially useful to test predefined species delimitation models or competing taxonomies, ranking models by marginal likelihood estimations (Baele et al., 2012), and using Bayes factors (Kass and Raftery, 1995) to assess support for model rankings. In order to run BFD analysis, a total of 28 samples were selected representing *B. bufo* species group and two different species arrangement models were constituted: 1) Current taxonomy was taken as reference model considering *B. bufo* and *B. verrucosissimus* as separate species (Recuero et al., 2012); 2) *B. verrucosissimus* was supposed to a subspecies of *B. bufo* (Garcia-Porta et al., 2012). The models were tested by calculating the marginal likelihood estimates (MLE) using stepping-stone sampling (Xie et al., 2011). Chain lengths were selected 10^6 , number of steps 100 and alpha value 0.3 and burn-in %10. After obtaining MLE values, the models were compared to calculate Bayes factor (BF) with the formula: $2 \times (\text{model1} - \text{model2})$ and crosschecked using the framework of BF support values (see Leache and Bouckaert, 2018).

Statistical analyses of morphological data

Prior to multivariate analyses, linear measurements were ln-transformed to reduce deviations from normality, the effect of variation in individual size, and to increase the fit to the requirements for such analyses (Sokal and Rohlf, 1981). As male and female toads differed markedly in body size, the two sexes were analyzed separately. We used discriminant analysis to quantify the morphometric differences among the genetically defined groups (see Results), herein referred to as *B. bufo*, 'verrucosissimus_North' and 'verrucosissimus_South'. We also applied cross-validation to estimate the predictive accuracy of morphological classification. To allow the application of diagnostic character states

in the field, measurements were analyzed with Student's *t*-test on selected untransformed data. Three derived characters of Arntzen et al. (2013b) were only used in univariate analyses. All statistical analyses were carried out with IBM SPSS v.21 (IBM Corp., 2012). Results were visualized with PAST 3.25 (Hammer et al., 2001).

Results

Molecular phylogenetics

For all 74 individuals studied we obtained 547 homologous base pairs for 16S, 786 bp for Cytb, 937 bp for RAG1 and 522 bp for RPL3. All newly generated DNA sequences have been deposited in GenBank (supplementary table S1). Aligning the sequences was straightforward but included a 25 bp indel for RPL3 that separated all individuals from localities 64-71 (Hatay, Mersin and Osmaniye) from the remaining ones, thus representing a putative molecular synapomorphy of the *B. verrucosissimus* South clade. For 16S, we found eight different haplotypes distributed in two groups separated by five substitutions; these groups represent *B. bufo* ($n = 6$, B1-B6) and *B. verrucosissimus* ($n = 2$, V1 and V2). For Cytb, we found 23 different haplotypes. These also classified in two groups that were separated by nine substitutions and represent *B. bufo* ($n = 18$, B7-B23 and B56), and *B. verrucosissimus* ($n = 5$, V3-V7). The haplotype network based on concatenated mitochondrial genes (fig. 1D) showed a clear separation between the two groups.

We found four different haplotypes for RPL3 and RAG1 nuclear genes that did not show full separation between species. The haplotype networks for RPL3 and RAG1 genes are shown in fig. 1B, C. The specimens of *B. verrucosissimus* from Antalya shared haplotype B54 with *B. bufo*, Artvin specimens shared haplotype V16 and the rest of *B. verrucosissimus* specimens from Mediterranean Region shared V17 haplotype for RPL3 gene. But Isparta specimens of *B. bufo* shared haplotype V15 with Mediterranean *B. verrucosissimus* specimens for RAG1 gene.

Finally, Artvin specimens of *B. verrucosissimus* shared haplotype B51 with *B. bufo*.

We recovered consistent topologies in BI and ML analyses of mtDNA sequences (fig. 3), with two major clades corresponding to *B. bufo* and *B. verrucosissimus*. According to these results, *B. verrucosissimus* is found in two disjunct regions: in the northeast of Turkey (Artvin) and along the Mediterranean coast in the south (Osmaniye, Hatay, Mersin and Antalya), while *B. bufo* is present at the remaining localities sampled. Within the *B. verrucosissimus* clade, a separate group including samples from Russia and the north of Georgia has strong support. Stronger support was found for two further subclades, referred to as *verrucosissimus_North* (including the V08 haplotype from Artvin and samples from the southwest of Georgia in localities Adjara and Borjomi) and *verrucosissimus_South* (including haplotypes V09-V12, found in the Turkish Mediterranean localities Osmaniye, Hatay, Mersin and Antalya).

The species trees obtained from the *BEAST analyses revealed generally fully resolved topologies and represented topological congruencies compared with the mitochondrial gene tree. The inferred species trees support the monophyly of *B. bufo* and *B. verrucosissimus* with high supported, with branch values having $BPP \geq 95$ (fig. 4). Furthermore, the concatenated species tree constructed from all loci produced identical multilocus species tree topology with approximate branch support values as in Recuero et al. (2012). The nuclear species tree also revealed similar results, except for a weakly supported *B. eichwaldi* branch, probably because of a lack of RAG1 data available for this taxon.

In the stepping-stone analysis, the MLE was found to be -5848.289 for model 1 (current taxonomy, i.e. considering *B. bufo* and *B. verrucosissimus* as separate species) and -5867.362 for model 2 (considering *B. verrucosissimus* as a subspecies of *B. bufo*). Subsequently, the BF

calculation was made against the current taxonomy model (model 1) and the value was calculated as $+39$ ($BF > 10$, decisive). The positive BF value indicated strong support for model 1, distinguishing *B. bufo* and *B. verrucosissimus* as distinct species. The result also matched with species trees those obtained in the *BEAST analysis.

Multivariate morphological differentiation

Discriminant functions separate the three genetically identified groups to various degrees. For females, the largest contributions to the first discriminant axis are by characters SVL, LPW, RADUL, and LHEAD, PDP and IOD, with negative and positive factor loads, respectively. At the second axis, the characters with a higher contribution are WHEAD, WGRASP and PDP (negative), and LTIB and EYETYM (positive). Scores of correct group classification were 97.9% for the original grouping and 86.5% for cross validation (supplementary table S2). For males, the largest contributions to the first axis are by characters RPW and SVL (negative) and LTARS (positive). At the second axis the characters contributing most are RPW, EYETYM, WHEAD (positive), and LPW and IOD (negative). Scores of correct group classification were 97.9% for the original grouping and 81.4% for cross validation (supplementary table S2). The results are summarized in fig. 5.

Univariate morphological differentiation of northern and southern B. verrucosissimus

The investigated character states followed a normal distribution (Kolmogorov-Smirnov test, $P > 0.05$). Student's *t*-tests identified some morphological differences between the two different groups of *B. verrucosissimus*. Descriptive statistics are presented in table 1: *verrucosissimus_South* toads have wider and more divergent parotoids than *verrucosissimus_North* toads, and the PA of *verrucosissimus_South* toads is smaller, congruently with PD for both sexes. Moreover, in males (but not in females),

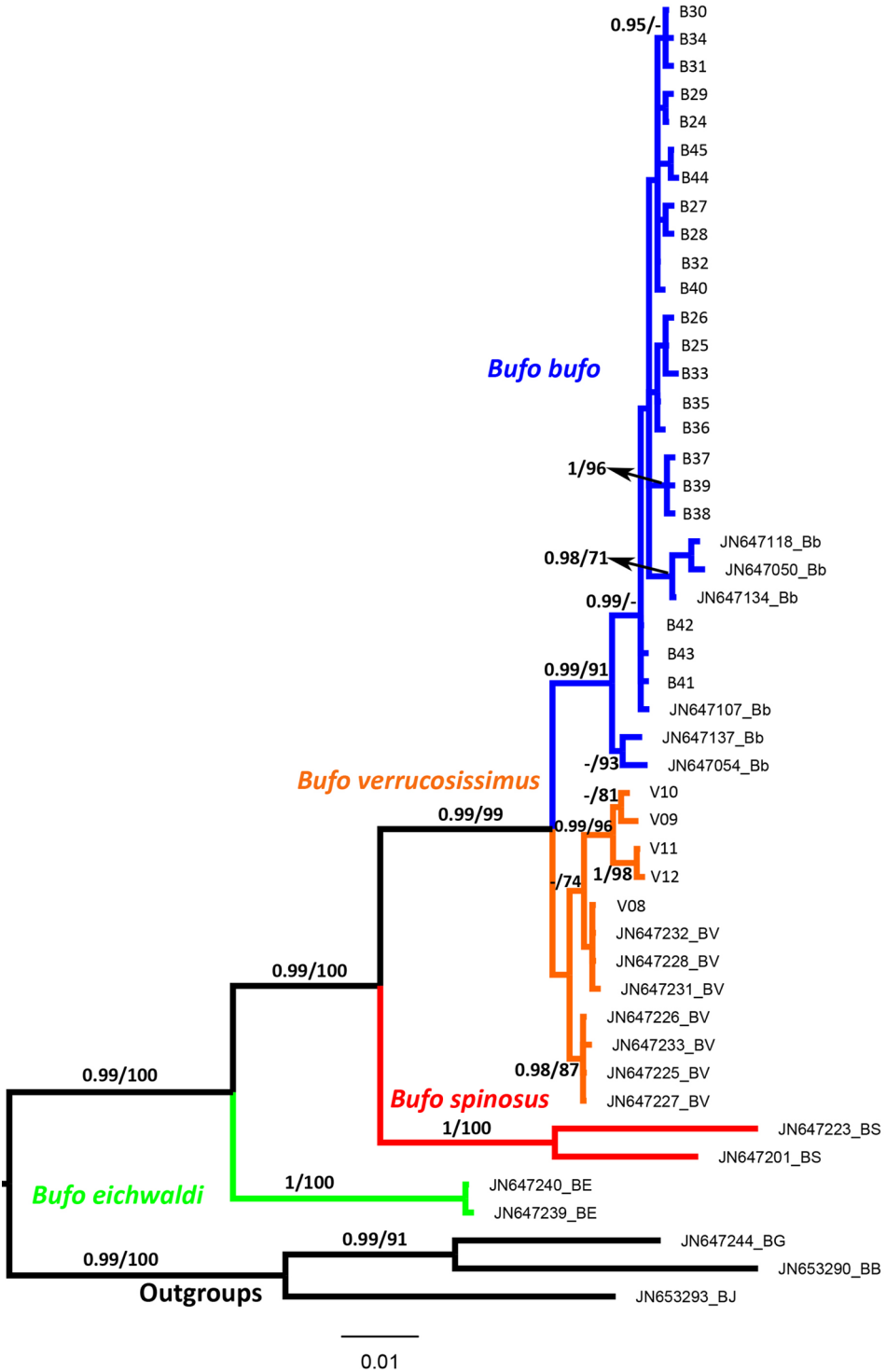


Figure 3. Phylogenetic tree based on Bayesian analysis of concatenated mtDNA sequences of the *Bufo bufo* species group. Colour codes as in fig. 1. Support values for internal nodes are on the zero to one scale for BI-analyses and from zero to 100 for ML-analyses. Hyphen (-) denotes <95% BPP and <70% ML bootstrap support values. Haplotype V08 represents *B. verrucosissimus* North while haplotypes V09-V012 represent *B. verrucosissimus* South.

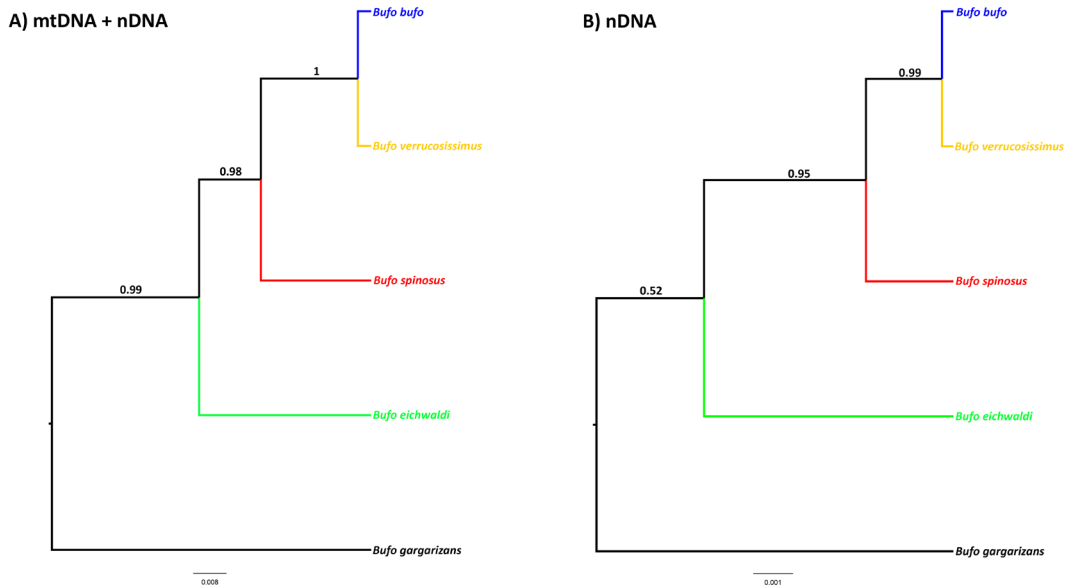


Figure 4. The species tree constructed using *BEAST. A: Concatenated dataset (all loci); B: Nuclear loci. Posterior probabilities are shown with support values.

in parallel with PD, PDA and especially PDP of *verrucosissimus*_South members are longer.

Discussion

Previous studies have shown *B. verrucosissimus* to occur in at least three different regions in Turkey (García-Porta et al., 2012; Recuero et al., 2012; Arntzen et al., 2013a). First, García-Porta et al. (2012) reported the occurrence of *B. verrucosissimus* at Lake Abant, in the north-western of Turkey, based on allozyme data (no mtDNA sequences were available). The reanalysis of this allozyme dataset by Arntzen et al. (2013a) suggested that the sample of Lake Abant analyzed by Garcia-Porta et al. (2012) was composed of one *B. verrucosissimus* and three *B. bufo*, with no evidence for admixture. Our study, based on both mtDNA and nDNA sequences, only found evidence for the presence of *B. bufo* in this area (figs 2 and 3). Considering the closest *B. verrucosissimus* populations are very distant (750 km, see fig. 3), we suggest that the reported presence of *B. verrucosissimus* in Lake Abant in previous studies may result from an error in sample labelling or processing.

Excluding the isolated Lake Abant record, *B. verrucosissimus* is present in two disjunct regions in Turkey. A first group is present in the South, along the Mediterranean coast, as first reported by Kutrup et al. (2006) based on the analysis of samples from Mersin. Later, Garcia-Porta et al. (2012) reported the presence of *B. verrucosissimus* in Alanya (their locality 167) based on both mitochondrial DNA sequences and allozymes (García-Porta et al., 2012). Our study further extends the range of *B. verrucosissimus* in southern Turkey with new locality records (supplementary table S1). This group of populations seems to be isolated by the Taurus Mountains to the north (fig. 1). These mountains have provided a complex topographic and microclimatic setting that has functioned as a historical refugium for the persistence of many amphibians (Gvozdik et al., 2010; Gül, 2013; Plötner et al., 2015; van Riemsdijk et al., 2017) and reptiles (Kapli et al., 2013; Tamar et al., 2015; Candan et al., 2016; Skourtanioti et al., 2016; Stümpel et al., 2016; Kotsakiozi et al., 2018). These populations of *B. verrucosissimus* seem to be isolated from those in Syria and Lebanon (see Jablonski and Sadek, 2019).

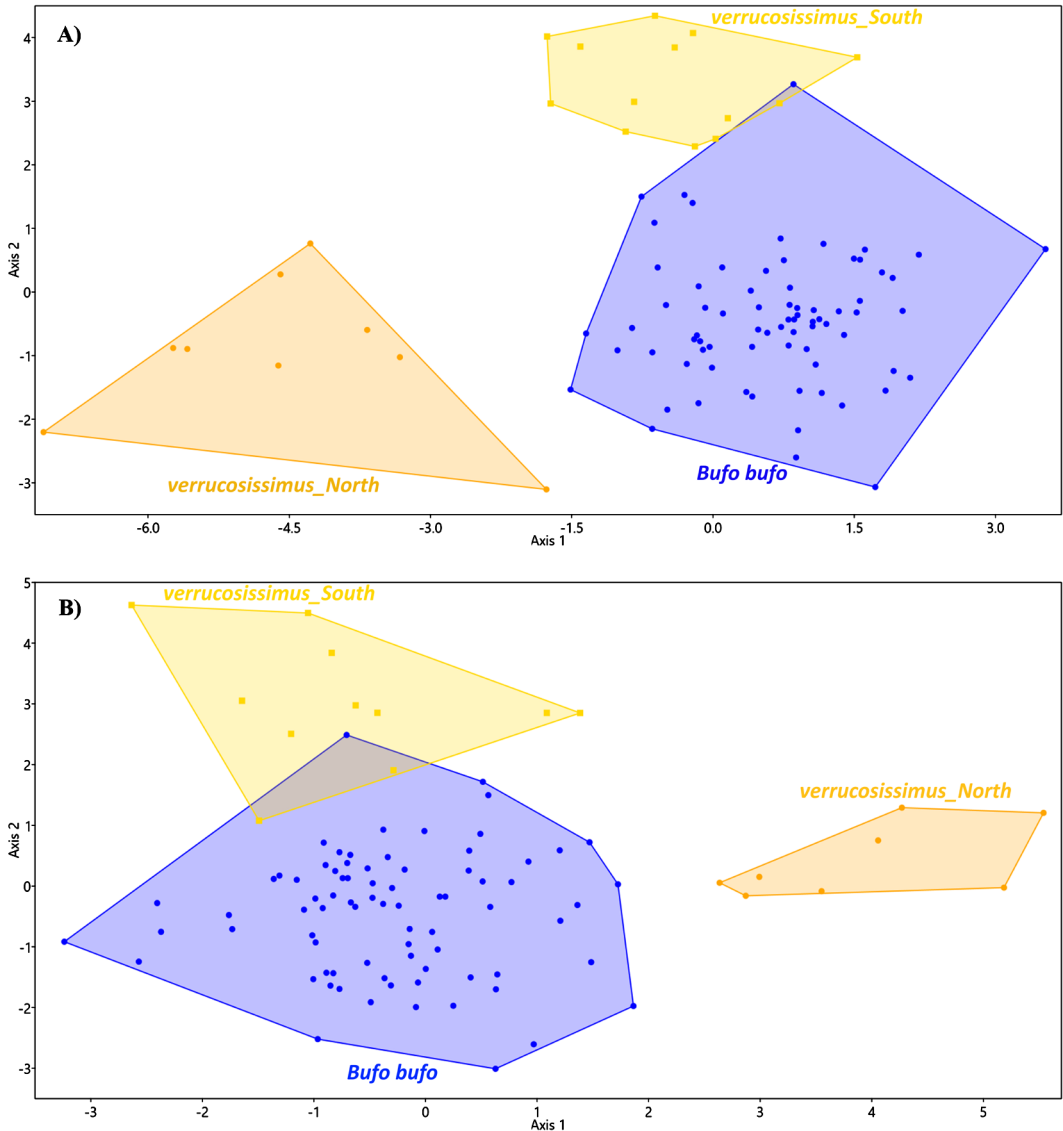


Figure 5. Bivariate plots of factor loadings on the first and second axes of the discriminant analysis based on morphological data for the three groups as identified by molecular analyses (fig. 2). A: Females; B: Males.

A second group of populations of *B. verrucosissimus* occupies northeastern Turkey and the Caucasus. Kutrup et al. (2006) did not find significant differences in mtDNA 16S sequences in samples from the Black Sea Region, Italy and Greece, including those from Artvin in Turkey, which were later assigned to *B. verrucosissimus* by Recuero et al. (2012), in agreement with our study. García-Porta et al. (2012) analyzed

a sample from a single locality from northeastern Turkey (Anayurt near Trabzon), which was assigned to *B. bufo* (García-Porta et al., 2012). This raises the possibility of the existence of a hybrid zone between *B. bufo* and *B. verrucosissimus* in this region, since according to Tunıyev et al. (2014), the range of *B. verrucosissimus* in Turkey extends from the west of Kaçkar Mountains to the boundary of Şavşat district, Artvin.

Table 1. Descriptive statistics of morphological variables (see text for abbreviations) and results of Student's *t*-test for differences between *B. verrucosissimus* North and *B. verrucosissimus* South.

Character	<i>B. verrucosissimus</i> North (mean ± SE)	<i>B. verrucosissimus</i> South (mean ± SE)	<i>t</i>	df	<i>P</i>
Females					
PA	60.2222 ± 3.08121	46.2308 ± 2.59228	3.467	20	<0.05
RPW	8.4233 ± 0.36885	10.1862 ± 0.43479	-2.901	20	<0.05
LPW	7.5800 ± 0.57895	10.0169 ± 0.44205	3.401	20	<0.05
PD	0.7076 ± 0.01408	0.6101 ± 0.01421	3.350	20	<0.01
Males					
PA	67.5000 ± 1.48805	49.3636 ± 3.52465	4.168	17	<0.05
RPW	5.3575 ± 0.23716	8.0055 ± 0.45154	-4.646	17	<0.01
LPW	5.2325 ± 0.17740	8.0055 ± 0.45154	5.003	17	<0.01
PD	0.7279 ± 0.01382	0.6534 ± 0.01601	3.467	17	<0.05
PDA	16.0563 ± 0.21598	18.8418 ± 0.83567	-2.773	17	<0.05
PDP	22.1188 ± 0.52675	29.1055 ± 1.64129	3.511	17	<0.05

Future studies should focus on this area and quantify patterns of reproductive isolation under a cline analysis framework as implemented in van Riemsdijk et al. (2019).

The disjunct distribution of *B. verrucosissimus* in Turkey, with populations in the Mediterranean and in the Caucasus parallels observations in other taxa, notably reptiles (Fritz et al., 2009; Jandzik et al., 2013; Milucek et al., 2013; Tamar et al., 2016), but also amphibians (van Riemsdijk et al., 2017; Dufresnes et al., 2019). A major biogeographic event potentially related with this peculiar pattern is the emergence of the Anatolian Diagonal, an important geographic barrier associated with changes in species diversity and composition (Davis, 1971; Nilson et al., 1990; Yiğit et al., 2012; Gül, 2013; Mohammadin et al., 2017; Şeker et al., 2018; Kočiš et al., 2018). The combination of mountain orogenesis and climatic changes during the Pleistocene may have driven the disjunct evolution of the two lineages of *B. verrucosissimus*, a divergence which seems recent, in view of the incomplete sorting of some nuclear alleles that are shared between the two populations as well as with some specimen of its sister taxon *B. bufo*.

Other amphibian taxa show diverged lineages along the Black Sea shore, as is observed in *Bufo*. For instance, *Triturus karelinii* is represented with three distinct gene pool groups along the Black Sea region (Wielstra and Arntzen, 2011; Wielstra et al., 2013a). Further studies revealed that these groups represent different species: *T. karelinii* sensu stricto, *T. anatolicus* and *T. ivanbureschi* (Wielstra et al., 2013b, 2014; Wielstra and Arntzen, 2016). In the north-east of Turkey, *T. karelinii* and *T. anatolicus* show a distribution gap. The genus *Lissotriton* shows a (wider) distribution gap in Anatolia as well, with *L. kosswigi* occurring in the west and *L. lantzi* protruding in the east (Wielstra et al., 2015, 2018). The occurrence of *B. bufo* and *verrucosissimus_North* along the Black Sea coast suggest a potential parallel biogeographic history. While there are no localities known where the two *Bufo* taxa meet, the distance between known *B. bufo* and *verrucosissimus_North* is smaller than observed in the two new genera and it is possible there is a secondary contact zone.

Discriminant analysis of morphological data showed separation among groups as defined by molecular analyses. The characters involved are related to variation in the parotoids, inner metatarsal tubercles and snouth-venth length.

Differences in parotoids and the inner metatarsal tubercle were also used as diagnostic characters to separate *B. bufo* and *B. spinosus* in previous studies (Arntzen et al., 2013b, 2014, 2016, 2017, 2018; Trujillo et al., 2017). We also found some differences between the two major *B. verrucosissimus* groups, with *verrucosissimus_South* individuals having more divergent parotoids than *verrucosissimus_North*. Observed differences, taking into account sexual dimorphism, may facilitate species assignment of adult toads in the field.

Through the combination of our comprehensive sampling, using different loci and Genbank data, the species status of *B. bufo* and *B. verrucosissimus* was supported. *B. verrucosissimus* was previously considered as a subspecies of *B. bufo* (Garcia-Porta et al., 2012), whereas following studies presented it as a separate species (Recuero et al., 2012; Arntzen et al., 2013b). A recent paper (van Riemsdijk et al., 2019; preprint on BioRxiv) shows that restriction-site associated DNA (RAD) sequencing data works well for the *Bufo* complex. So future genome-scale analysis (such as RADseq) would be a valuable next step for working on contact zones. In addition, more detailed analyses focusing on reproductive isolation in contact zones (both in north and south of Turkey) using other markers (like microsatellites or SNPs) and under a cline analysis framework, need to be carried out to settle the issue.

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References

- Agasyan, A., Avisi, A., Tuniyev, B., Crnobrnja-Isailovic, J., Lymberakis, P., Andrén, C., Cogalniceanu, D., Wilkinson, J., Ananjeva, A., Üzümlü, N., Orlov, N., Podlousky, R., Tuniyev, S., Kaya, U. (2009): *Bufo bufo*. The IUCN Red List of Threatened Species 2009: e.T54596A11159464.
- AmphibiaWeb (2016): *Bufo eichwaldi*: Eichwald's toad. University of California, Berkeley, CA. <http://amphibiaweb.org/species/7152> (accessed 18 September 2019).
- Arntzen, J.W., Recuero, E., Canestrelli, D., Martínez-Solano, I. (2013a): How complex is the *Bufo bufo* species group? *Mol. Phylogenet. Evol.* **69**: 1203-1208.
- Arntzen, J.W., Wilkinson, J.W., Butôt, R., Martínez-Solano, I. (2014): A new vertebrate species native to the British Isles: *Bufo spinosus* Daudin, 1803 in Jersey. *Herpetol. J.* **24**: 209-216.
- Arntzen, J.W., Trujillo, T., Butot, R., Vrieling, K., Schaap, O., Rodriguez, J.G., Martínez-Solano, I. (2016): Concordant morphological and molecular clines in a contact zone of the common and spined toad (*Bufo bufo* and *B. spinosus*) in the northwest of France. *Front. Zool.* **13**: 52.
- Arntzen, J.W., De Vries, W., Canestrelli, D., Martínez-Solano, I. (2017): Hybrid zone formation and contrasting outcomes of secondary contact over transect in common toads. *Mol. Ecol.* **26**: 5663-5675.
- Arntzen, J.W., McAtear, J., Butôt, R., Martínez-Solano, I. (2018): A common toad hybrid zone that runs from the Atlantic to the Mediterranean. *Amph. Rept.* **39**: 41-50.
- Arntzen, W.J., McAtear, J., Recuero, E., Ziermann, J.M., Ohler, A., Van Alphen, J., Martínez-Solano, I. (2013b): Morphological and genetic differentiation of *Bufo* toads: two cryptic species in western Europe (Anura, Bufonidae). *Contrib. Zool.* **82**: 147-169.
- Baele, G., Lemey, P., Bedford, T., Rambaut, A., Suchard, M.A., Alekseyenko, A.V. (2012): Improving the accuracy of demographic and molecular clock model comparison while accommodating phylogenetic uncertainty. *Mol. Biol. Evol.* **29** (9): 2157-2167.
- Borchsenius, F. (2009): FastGap 1.2. Distributed by the author. http://192.38.46.42/aubot/fb/FastGap_home.htm (accessed 10 May 2011).
- Bouckaert, R., Vaughan, T.G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., et al. (2019): BEAST 2.5: an advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* **15** (4): e1006650.
- Bouckaert, R., Drummond, A. (2017): bModelTest: Bayesian phylogenetic site model averaging and model comparison. *BMC Evol. Biol.* **17**: 42. DOI:10.1186/s12862-017-0890-6.
- Candan, K., Kankılıç, T., Güçlü, Ö., Kumluca, Y., Durmuş, S.H., Lymberakis, P., Poulakakis, N., Ilgaz, Ç. (2016): First assessment on the molecular phylogeny of *Anatololacerta* (Squamata, Lacertidae) distributed in southern Anatolia: insights from mtDNA and nDNA markers. *Mitochondrial DNA A* **27**: 2285-2292.

- Castellano, S., Giacoma, C. (1998): Morphological variation of the green toad, *Bufo viridis*, in Italy: a test of causation. *J. Herpetol.* **32**: 540-550.
- Cossu, I.M., Frau, S., Delfino, M., Chiodi, A., Corti, C., Bellati, A. (2018): First report of *Bufo bufo* (Linnaeus, 1758) from Sardinia (Italy). *Acta. Herpetol.* **13** (1): 43-49.
- Davis, P.H. (1971): Distribution patterns in Anatolia with particular reference to endemism. In: *Plant Life of South-West Asia*, p. 15-27. Davis, P.H., Harper, P.C., Hedge, I.C., Eds, Bot. Society of Edinburgh, Edinburgh.
- Dufresnes, C., Strachinis, I., Suriadna, N., Myktyynets, G., Cogălniceanu, D., Székely, P., Vukov, T., Arntzen, J.W., Wielstra, B., Lymberakis, P., Geffen, E., Gafny, S., Kumlutaş, Y., Ilgaz, Ç., Candan, K., Mizsei, E., Szabolcs, M., Kolenda, K., Smirnov, N., Géniez, P., Lukanov, S., Crochet, P.-A., Dubey, S., Perrin, N., Litvinchuk, S.N., Denoël, M. (2019): Phylogeography of a cryptic speciation continuum in Eurasian spadefoot toads (*Pelobates*). *Mol. Ecol.* **28**: 3257-3270.
- Feng, Y.J., Blackburn, D.C., Liang, D., Hillis, D.M., Wake, D.B., Cannatella, D.C., Zhang, P. (2017): Phylogenomics reveals rapid, simultaneous diversification of three major clades of Gondwanan frogs at the Cretaceous-Paleogene boundary. *PNAS* **114**: E5864-E5870.
- Fritz, U., Harris, D.J., Fahd, S., Rouag, R., Martínez, E.G., Casaldueiro, A.G., Široký, P., Kalboussi, M., Jdeidi, T.B., Hundsdoerfer, A.K. (2009): Mitochondrial phylogeography of *Testudo graeca* in the western Mediterranean: old complex divergence in north Africa and recent arrival to Europe. *Amph. Rept.* **30**: 63-80.
- Frost, D.R. (2019): Amphibian species of the world: an online reference. Version 6.0 (date of access). Electronic database accessible at <http://research.amnh.org/herpetology/amphibia/index.html>. American Museum of Natural History, New York, USA.
- García-Porta, J., Litvinchuk, S.N., Crochet, P.A., Romano, A., Geniez, P.H., LoValvo, M., Lymberakis, P., Carranza, S. (2012): Molecular phylogenetics and historical biogeography of the west-palaearctic common toads (*Bufo bufo* species complex). *Mol. Phylogenet. Evol.* **63**: 113-130.
- Goebel, A.M., Donnelly, J.M., Atz, M.E. (1999): PCR primers and amplification methods for 12S ribosomal DNA, the control region, cytochrome oxidase I, and cytochrome b in bufonids and other frogs, and an overview of PCR primers which have amplified DNA in amphibians successfully. *Mol. Phylogenet. Evol.* **11**: 163-199.
- Gül, S. (2013): Ecological divergence between two evolutionary lineages of *Hyla savignyi* (Audouin, 1827) in Turkey: effects of the Anatolian Diagonal. *Anim. Biol.* **63**: 285-295.
- Gvozdič, V., Moravec, J., Klütsch, C., Kotlík, P. (2010): Phylogeography of the Middle Eastern tree frogs (*Hyla*, Hylidae, Amphibia) as inferred from nuclear and mitochondrial DNA variation, with a description of a new species. *Mol. Phylogenet. Evol.* **55**: 1146-1166.
- Hall, T.A. (1999): BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* **41**: 95-98.
- Hammer, Ø., Harper, D.A.T., Ryan, P.D. (2001): PAST: paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* **4** (1): 4.
- IBM Corporation (2012): IBM SPSS statistics for Windows, version 21.0. IBM Corporation, New York.
- Jablonski, D., Sadek, R.A. (2019): The Caucasian toad, *Bufo verrucosissimus* (Pallas, 1814) in the Levant: evidence from mitochondrial DNA. *Herpetozoa* **32**: 255-258. DOI:10.3897/herpetozoa.32.e37560.
- Jandzik, D., Avcı, A., Gvozdič, V. (2013): Incongruence between taxonomy and genetics: three divergent lineages within two subspecies of the rare Transcaucasian rat snake (*Zamenis hohenackeri*). *Amph. Rept.* **34**: 579-584.
- Kaplı, P., Botoni, D., Ilgaz, Ç., Kumlutaş, Y., Avcı, A., Pouyani, N.R., Fathinia, B., Lymberakis, P., Ahmadzadeh, F., Poulakakis, N. (2013): Molecular phylogeny and historical biogeography of the Anatolian lizard *Apathya* (Squamata, Lacertidae). *Mol. Phylogenet. Evol.* **66**: 992-1001.
- Kass, R.E., Raftery, A.E. (1995): Bayes factors. *J. Am. Stat. Assoc.* **90**: 773-795.
- Kočiš Tubić, N., Stáhl, G., Ačanski, J., Djan, M., Viđaković, D.O., Hayat, R., Khaghaninia, S., Vujić, A., Radenković, S. (2018): An integrative approach in the assessment of species delimitation and structure of the *Merodon nanus* species group (Diptera: Syrphidae). *Org. Divers. Evol.* **18**: 479-497.
- Kotsakiozi, P., Jablonski, D., Ilgaz, Ç., Kumlutaş, Y., Avcı, A., Meiri, S., Itescu, Y., Kukushkin, O., Gvozdič, V., Scillitani, G., Roussos, S.A., Jandzik, D., Kasapidis, P., Lymberakis, P., Poulakakis, N. (2018): Multilocus phylogeny and coalescent species delimitation in Kotschy's gecko, *Mediodactylus kotschyi*: hidden diversity and cryptic species. *Mol. Phylogenet. Evol.* **125**: 177-187.
- Kutrup, B., Yılmaz, N., Canakci, S., Belduz, A.O., Doglio, S. (2006): Intraspecific variation of *Bufo bufo*, based on 16S ribosomal RNA sequences. *Amph. Rept.* **27**: 268-273.
- Leaché, A.D., Bouckaert, R.R. (2018): Species trees and species delimitation with SNAPP: a tutorial and worked example. In: *Workshop on Population and Speciation Genomics*, Český Krumlov.
- Leigh, J.W., Bryant, D. (2015): POPART: full-feature software for haplotype network construction. *Methods Ecol. Evol.* **6**: 1110-1116.
- Liedtke, H.C., Muller, H., Hafner, J., Penner, J., Gower, D.J., Mazuch, T., Rodel, M.O., Loader, S.P. (2017): Terrestrial reproduction as an adaptation to steep terrain in African toads. *Proc. R. Soc. B* **284** (1851): 20162598.
- Litvinchuk, N.S., Borkin, L., Skoronov, V.D., Rosanov, J.M. (2008): A new species of common toads from the Talysh mountains, south-eastern Caucasus: genome size, allozyme, and morphological evidences. *Russ. J. Herpetol.* **15**: 19-43.
- Mertens, R., Wermuth, H. (1960): *Die Amphibien und Reptilien Europas*. Verlag Valdemar Kramer, Frankfurt.
- Mikulicek, P., Jandzik, D., Firtz, U., Schneider, C., Siroky, P. (2013): AFLP analysis shows high incongruence between genetic differentiation and morphology-based taxonomy in a widely distributed tortoise. *Biol. J. Linn. Soc.* **108**: 151-160.

- Mohammadin, S., Peterse, K., van de Kerke, S.J., Chattrou, L.W., Dönmez, A.A., Mummenhoff, K., Pires, J.C., Edger, P.P., Al-Shehbaz, I.A., Schranz, M.E. (2017): Anatolian origins and diversification of *Aethionema*, the sister lineage of the core Brassicaceae. *Am. J. Bot.* **104**: 1042-1054.
- Nilson, G., Andrén, C., Flärdh, B. (1990): *Vipera albizona*, a new mountain viper from central Turkey, with comments on isolating effects of the Anatolian "Diagonal". *Amph. Rept.* **11**: 285-294.
- Nylander, J.A.A. (2004): MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Orlova, V.F., Tuniyev, B.S. (1989): On the taxonomy of the Caucasian common toads belonging to the group *Bufo bufo verrucosissimus* (Pallas) (Amphibia, Anura, Bufonidae). *Bull. Mosk. Obshch. Isp. Prirody Ser. Biol.* **94**: 13-24.
- Palumbi, S.R., Martín, A., Romano, S., McMillan, W.O., Stice, L., Grabowski, G. (1991): Simple fool's guide to PCR, version 2.0. University of Hawaii, Honolulu, Hawaii.
- Plötner, J., Pekşen, Ç.A., Baier, F., Uzzell, T., Bilgin, C.C. (2015): Genetic evidence for human-mediated introduction of Anatolian water frogs (*Pelophylax cf. bedriagae*) to Cyprus (Amphibia: Ranidae). *Zool. Middle East* **61**: 125-132.
- Pramuk, J.B. (2006): Phylogeny of South American *Bufo* (Anura: Bufonidae) inferred from combined evidence. *Zool. J. Linn. Soc.* **146**: 407-452.
- Rambaut, A. (2012): FigTree 1.4.0 software. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh.
- Rambaut, A., Drummond, A.J., Xie, D., Baele, G., Suchard, M.A. (2018): Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Syst. Biol.* **67**: 901-904.
- Recuero, E., Canestrelli, D., Vörös, J., Szabó, K., Poyarkov, N.A., Arntzen, J.W., Crnobrnja-Isailovic, J., Kidov, A.A., Cogălniceanu, D., Caputo, F.P., Nascetti, G., Martínez-Solano, I. (2012): Multilocus species tree analyses resolve the radiation of the widespread *Bufo bufo* species group (Anura, Bufonidae). *Mol. Phylogenet. Evol.* **62**: 71-86.
- Ronquist, F., Huelsenbeck, J.P. (2003): MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572-1574.
- San Mauro, D., García-París, M., Zardoya, R. (2004): Phylogenetic relationships of discoglossid frogs (Amphibia: Anura: Discoglossidae) based on complete mitochondrial genomes and nuclear genes. *Gene* **343**: 357-366.
- Şeker, S.P., Selvi, E., Kankılıç, T., Çolak, E. (2018): Geographical distribution pattern of mitochondrial DNA cytochrome b diversity in populations of *Arvicola amphibius* (Linnaeus, 1758) (Mammalia: Rodentia) in Turkey as determined by PCR-RFLP. *Acta Zool. Bulg.* **70**: 19-30.
- Sinsch, U., Schneider, H., Tarkhishvili, D.N. (2009): *Bufo bufo* Superspezies – Erdkröten-Artenkreis – taxon *bufo* (Linnaeus, 1758) – Erdkröte – taxon *gredosicola* L. Müller and Hellmich, 1935 – Gredoserdkröte – taxon *spinosus* Daudin, 1803 – Riesenerdkröte – taxon *verrucosissimus* (Pallas, 1811) – Kolchische Erdkröte. In: *Handbuch der Reptilien und Amphibien Europas*. Froschlurche II, p. 191-335. Grossenbacher, K., Ed., Aula Verlag, Wiebelsheim.
- Skourtanoti, E., Kapli, P., Ilgaz, Ç., Kumlutaş, Y., Avcı, A., Ahmadzadeh, F., Isailovic, J.C., Gherghel, I., Lymberakis, P., Poulakakis, N. (2016): A reinvestigation of phylogeny and divergence times of the *Ablepharus kitaibelii* species complex (Sauria, Scincidae) based on mtDNA and nuDNA genes. *Mol. Phylogenet. Evol.* **103**: 199-214.
- Sokal, R.R., Rohlf, F.J. (1981): *Biometry*, 2nd Edition. Freeman Company, San Francisco, CA.
- Stamatakis, A. (2014): RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**: 1312-1313.
- Stümpel, N., Rajabizadeh, M., Avcı, A., Wüster, W., Joger, U. (2016): Phylogeny and diversification of mountain vipers (*Montivipera*, Nilson et al., 2001) triggered by multiple Plio-Pleistocene refugia and high-mountain topography in the Near and Middle East. *Mol. Phylogenet. Evol.* **101**: 336-351.
- Tamar, K., Carranza, S., in den Bosch, H., Sindaco, R., Moravec, J., Meiri, S. (2015): Hidden relationships and genetic diversity: molecular phylogeny and phylogeography of the Levantine lizards of the genus *Phoenicolacerta* (Squamata: Lacertidae). *Mol. Phylogenet. Evol.* **91**: 86-97.
- Tamar, K., Smid, J., Göçmen, B., Meiri, S., Carranza, S. (2016): An integrative systematic revision and biogeography of *Rhynchocalamus* snakes (Reptilia, Colubridae) with a description of a new species from Israel. *PeerJ* **4**: e2769.
- Trujillo, T., Gutiérrez-Rodríguez, J., Arntzen, J.W., Martínez-Solano, I. (2017): Morphological and molecular data to describe a hybrid population of the common toad (*Bufo bufo*) and the spined toad (*Bufo spinosus*) in western France. *Contrib. Zool.* **86**: 1-10.
- Tuniev, B.S., Tuniev, S.B., Avcı, A., Ilgaz, Ç. (2014): Herpetological studies in eastern and north-eastern Turkey. *Curr. Herpetol.* **14** (1/2): 44-53 (in Russian).
- Van Bocxlaer, I., Biju, S.D., Loader, S.P., Bossuyt, F. (2009): Toad radiation reveals into India dispersal as a source of endemism in the Western Ghats Sri Lanka biodiversity hotspot. *BMC Evol. Biol.* **9**: 131.
- van Riemsdijk, I., Arntzen, J.W., Bucciarelli, G., McCartney-Melstad, E., Rafajlović, M., Scott, P.A., Toffelmier, E., Shaffer, H.B., Wielstra, B. Spatial variation in introgression along a toad hybrid zone in France. *bioRxiv* 746073. DOI:10.1101/746073.
- van Riemsdijk, I., Arntzen, J.W., Bogaerts, S., Franzen, M., Litvinchuk, S.N., Olgun, K., Wielstra, B. (2017): The Near East as a cradle of biodiversity: a phylogeography of banded newts (genus *Ommatotriton*) reveals extensive inter- and intraspecific genetic differentiation. *Mol. Phylogenet. Evol.* **114**: 73-81.
- van Riemsdijk, I., Butlin, R.K., Wielstra, B., Arntzen, J.W. (2019): Testing an hypothesis of hybrid zone movement for toads in France. *Mol. Ecol.* **28** (5): 1070-1083.

- Wielstra, B., Arntzen, J.W. (2011): Unraveling the rapid radiation of crested newts (*Triturus cristatus* superspecies) using complete mitogenomic sequences. *BMC Evol. Biol.* **11**: 162.
- Wielstra, B., Litvinchuk, S., Naumov, B., Tzankov, N., Arntzen, J.W. (2013a): A revised taxonomy of crested newts in the *Triturus karelinii* group (Amphibia: Caudata: Salamandridae), with the description of a new species. *Zootaxa* **3682**: 441-453.
- Wielstra, B., Baird, A.B., Arntzen, J.W. (2013b): A multimer phylogeography of crested newts (*Triturus cristatus* superspecies) reveals cryptic species. *Mol. Phylogenet. Evol.* **67**: 167-175.
- Wielstra, B., Sillero, N., Vörös, J., Arntzen, J.W. (2014): The distribution of the crested and marbled newt species (Amphibia: Salamandridae: *Triturus*) – an addition to the New Atlas of Amphibians and Reptiles of Europe. *Amph. Rept.* **35**: 376-381.
- Wielstra, B., Bozkurt, E., Olgun, K. (2015): The distribution and taxonomy of *Lissotriton* newts (Amphibia, Salamandridae) in Turkey. *ZooKeys* **484**: 11-23.
- Wielstra, B., Arntzen, J.W. (2016): Description of a new species of crested newt, previously subsumed in *Triturus ivanbureschi* (Amphibia: Caudata: Salamandridae). *Zootaxa* **4109**: 73-80.
- Wielstra, B., Canestrelli, D., Cvijanović, M., Denoël, M., Fijarczyk, A., Jablonski, D., Liana, M., Naumov, B., Olgun, K., Pabijan, M., Pezzarossa, A., Popgeorgiev, G., Salvi, D., Si, Y., Sillero, N., Sotiropoulos, K., Zieliński, P., Babik, W. (2018): The distributions of the six species constituting the smooth newt species complex (*Lissotriton vulgaris* sensu lato and *L. montandoni*) – an addition to the New Atlas of Amphibians and Reptiles of Europe. *Amph. Rept.* **39**: 252-259.
- Xie, W., Lewis, P.O., Fan, Y., Kuo, L., Chen, M.H. (2011): Improving marginal likelihood estimation for Bayesian phylogenetic model selection. *Syst. Biol.* **60**: 150-160.
- Yiğit, N., Markov, G., Çolak, E., Kocheva, M., Saygılı, F., Yüce, D., Çam, P. (2012): Phenotypic features of the ‘*guentheri*’ group vole (Mammalia: Rodentia) in Turkey and southeast Bulgaria: evidence for its taxonomic detachment. *Acta Zool. Bulg.* **64**: 23-32.

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