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Taxonomic status and distribution of common toads in Iran

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We report several new localities of common toads from northern Iran. Based on the study of external morphology and a fragment of the mitochondrial 16S rRNA gene, the Iranian common toads were identified as *Bufo eichwaldi*. Maximum entropy modelling was used to estimate the effects of altitude, precipitation and temperature on the distribution of *B. eichwaldi*. The distribution of the species is linked to a forest zone on the slopes of the Talysh and Elburz mountains.

Key words: Bufo eichwaldi, Environmental modelling, mtDNA

The common toads comprise two groups: the *Bufo gargarizans* complex distributed in the Eastern Palearctic and the *B. bufo* complex which occurs in the Western Palearctic (Garcia-Porta et al., 2012). The Eichwald's toad, *B. eichwaldi* Litvinchuk, Borkin, Skorinov & Rosanov, 2008, is restricted to the Talysh Mountains (Azerbaijan), while the European common toad, *B. bufo* (Linnaeus, 1758), expands from North Africa to the Polar circle and from Iberian Peninsula to Lake Baikal in Siberia. Some studies (e.g., Recuero et al., 2012) recognize two additional species within *B. bufo*: *B. verrucosissimus* (Pallas, 1814), which inhabits the Caucasus and Anatolia, and *B. spinosus* Mertens, 1925, from western and central France, the Iberian Peninsula and North Africa.

Blanford (1876) was the first who reported common toads (mentioned as *Bufo vulgaris*) for the territory of Iran, based on a wrong translation of P. S. Pallas's (1831) original Latin text: "Non deest in Persia…" (i.e. is lacking in Persia…). The first specimen of "*Bufo bufo bufo*" from Iran was collected between 1861 and 1908 by L. F. Mlokosevich from Shefid Rud (=Shaferuz) River and was kept in collections of the Caucasian State Museum, Tbilisi, Georgia (Zhordaniya, 1960). Three additional records of the common toads were described in the Mazandaran, Gilan and Golestan provinces of Iran (Eiselt & Schmidtler, 1973; Tuck, 1975; Kami & Vakilpoure, 1996).

So far the taxonomic status of common toads from Iran has been unclear. Different authors designated these toads either as *B. bufo* or *B. verrucosissimus* (Zhordaniya, 1960; Eiselt & Schmidtler, 1973; Tuck, 1975; Baloutch & Kami, 1995; Kami & Vakilpoure, 1996). Based on distribution pattern of the recently described *B. eichwaldi*, Litvinchuk et al. (2008) suggested that its range expands to northern Iran. The aim of the present work is to evaluate the taxonomic status and distribution patterns of common toads from Iran.

We analyzed a fragment of the mitochondrial 16S rRNA gene (601–631 bp), sequenced from a specimen from Iran (Shirabad) (Table 1), three specimens from the type locality of *B. eichwaldi* (Azfilial: 38.6500°N 48.8000°E), four specimens of *B. b. bufo* from Croatia (Ponikve Lake, Krk Island: 45.0781°N 14.5648°E) and Ukraine (Vilkovo: 45.3970°N 29.6073°E), two specimens of *B. b. verrucosissimus* from Russia (Kyurdzhinovo: 45.4167°N 40.6167°E) and Abkhazia (Lidzava: 43.1833°N 40.3500°E) and a specimen of *B. gargarizans* from Russia (Bonevurovka: 43.7167°N, 132.0333°E). The protocols for DNA extraction, amplification, and sequencing were previously described in Litvinchuk et al. (2011). The sequences were deposited to GenBank under accession numbers JX218095–JX218105.

A maximum likelihood (ML) tree was generated using PhyML version 2.4.5 (Guindon & Gascuel, 2003) under a Tamura 3-parameter model. The best fit model of nucleotide evolution was determined under the Akaike information criterion in jModeltest (Posada, 2008). We generated bootstrap values based on 1000 resampled data sets. *Bufo gargarizans* was used as an outgroup.

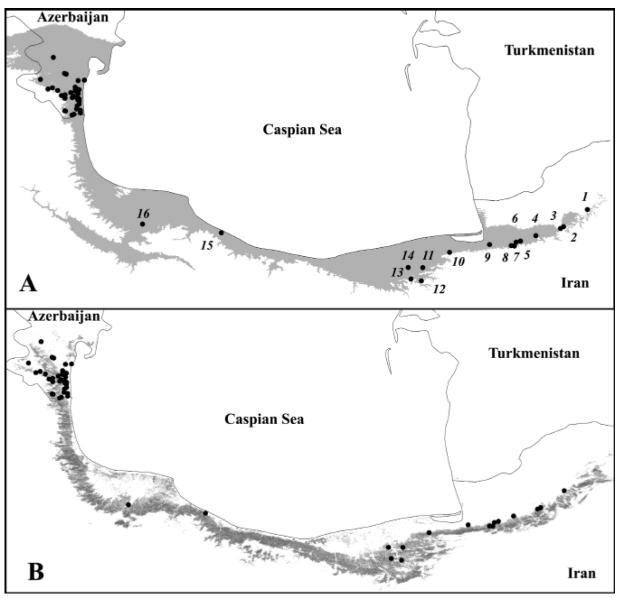

Fig. 1. Predicted potential distribution model (gray area) of *Bufo eichwaldi* made using MAXENT (*A*) and map of distribution of forests (gray area) in northern Iran (*B*). Numbers for Iranian localities are given in Table 1.

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Table 1. List of localities of *Bufo eichwaldi* in Iran. Alt is altitude (in m a.s.l.).

N	Locality	Latitude	Longitude	Alt	Reference
1	Minudasht	37.2176	55.3721	135	Our data
2	Sefidcheshmeh	36.9942	55.0648	106	ZMGU.2402
3	Shirabad	36.9725	55.0253	90	Our data
4	Ghorogh Jungle	36.9000	54.7333	140	Kami & Vakilpoure (1996)
5	Forest of Baghe Golbon	36.8112	54.5106	415	ZMGU.2280
6	Alang Darreh Pond	36.7965	54.4546	303	ZMGU.449, ZMGU.450
7	10 km south of Gorgan	36.7466	54.4335	690	ZMGU.1904, ZMGU.2548
8	Forest of Sasht Kalateh	36.7519	54.3907	519	ZMGU.2405
9	Kordkoy	36.7649	54.1110	59	ZMGU.2306
10	Abbas Abad pond	36.6635	53.5943	388	Our data
11	Ojarostagh (=Midanak)	36.2972	53.2318	593	ZMGU.412, ZMGU.413
12	20 km south-south-east of Sari	36.4667	53.2500	532	Tuck (1975)
13	26 km south of Sari	36.3210	53.0970	616	ZMGU.288, ZMGU.289
14	10 km south of Sari	36.4689	53.0621	400	ZMGU.315
15	10 km west of Ramsar	36.9167	50.6500	6	Eiselt & Schmidtler (1973)
16	Shefid Rud (=Shaferuz) River	37.0297	49.6346	84	Zhordaniya (1960)

The distribution of *B. eichwaldi* was modeled using 16 Iranian and 36 Azerbaijan georeferenced localities (Fig. 1). The geospatial layers were downloaded from the WorldClim 1.4 database (1950–2000; ~1 km resolution; http://www.worldclim.org/current). The distributional model for *B. eichwaldi* was generated by MAXENT 3.3.3k (Phillips & Dudík, 2008). MAXENT was used with default settings (Convergence threshold= 0.00001, maximum number of iterations=500 and β_j=0), partitioning the geographical records between training and test samples (75% and 25% respectively). In order to avoid highly correlated and redundant climate variables in our dataset, we estimated the correlation between pairs of variables using the Pearson correlation coefficient in ENMTOOLS 1.3 (Warren et al., 2008). Variables sharing a correlation coefficient of at least 0.80 were considered highly correlated. Five bioclimatic variables were retained: Bio6 (minimal temperature of coldest month; ºC), Bio8 (mean temperature of wettest quarter; ºC), Bio9 (mean temperature of driest quarter; ºC), Bio11 (mean temperature of coldest quarter; ºC), and Bio12 (annual precipitation; mm). Finally, the model was reclassified in ARCGIS 10 into the binary presence-absence map for which the average ten-percentile threshold was used (Raes et al., 2009).

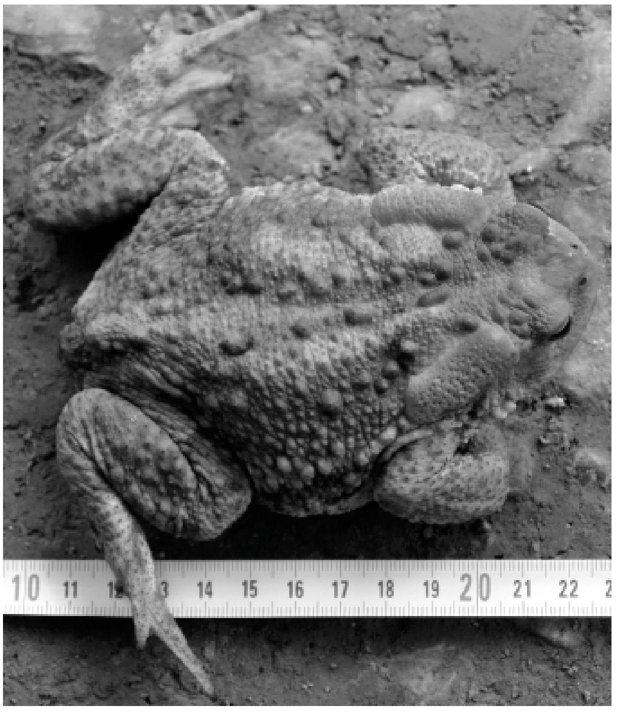

Fig. 2. *Bufo eichwaldi* from Shirabad.

A map of distribution of forests in the northern part of Iran (Fig. 1) was constructed by use of a geospatial layer downloaded from the GLCF database (2000–2001; ~0.5 km resolution; www.landcover.org; Hansen et al., 2002).

A female common toad (Fig. 2) was found by M. Auer (May 12, 2006) on a small road between Shirabad and Khan Bebin in Golestan Province (Table 1). In 2011, common toads were registered in Minoudasht Town in Golestan Province. In February 13, 2012, ovipositing common toads were observed by H.G. Kami in Abbas Abad pond near Behshahr city in Mazandaran Province. Additionally, 4 males, 6 females and 4 subadult common toads from nine new localities (Table 1) are preserved in the Zoological Museum of Golestan University (ZMGU), Gorgan, Iran.

All adult toads studied by us (4 males and 7 females) had a wide and massive head, with an abrupt snout tip in females. The dorsum of females was dull brown with darker free designs and rare small black spots. A lower parotid margin was contacting with the tympanum, bearing black markings. The toads had large rounded knobs on their dorsum (Fig. 2).

Molecular studies showed previously that *B. eichwaldi* forms a clade within the *B. bufo* species complex (Litvinchuk et al., 2008; Pisanets et al., 2009; Recuero et al., 2012; Garcia-Porta et al., 2012). However, so far Iranian common toads were unconsidered in phylogenetic studies. Our phylogenetic analysis distinguished two well supported clades corresponding to *B. bufo* and

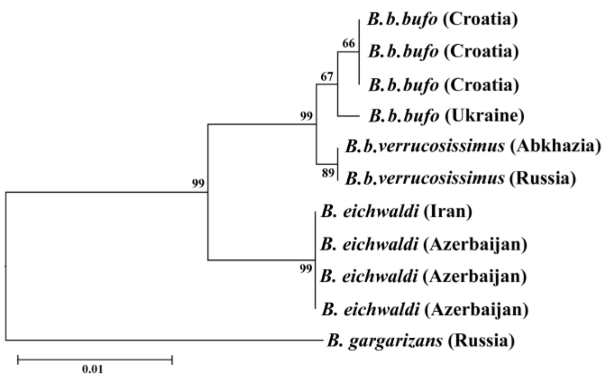

Fig. 3. Maximum likelihood phylogram (16S rRNA) of the species comprising the group of common toads. Numbers at nodes are bootstrap values in percent. The scale bar indicates amount of substitutions per site.

B. eichwaldi, the latter including the sample from Shirabad (Fig. 3). The obtained sequence was identical with the topotypic specimens of *B. eichwaldi*. The similarity of morphological characters, such as head and snout tip shape, dorsum coloration and existence of large rounded knobs, further allowed us to identify the common toads from Iran as *B. eichwaldi*.

The distribution model of *B. eichwaldi* had a high mean test AUC value (0.995±0.002) and showed significance for the binomial omission test, indicating a good performance of the model (Fig. 1). The relative contributions to the model of variables Bio12, Bio9, Bio6 and Bio8 were high (32, 27, 26, and 15% respectively). The range of Eichwald's toad in Iran is linked to a forest zone on slopes (up to 690 m a.s.l. in Iran) of the Talysh and Elburz mountains. *Bufo eichwaldi* is a nocturnal and secretive species which prefers habitats in foothills and low mountains with broad-leaved forests mixed with patches of grasslands, wetlands or agricultural plantations. *Hyla orientalis gumilevski* Litvinchuk, Borkin, Rosanov & Skorinov, 2008 and *Rana macrocnemis pseudodalmatina* Eiselt & Schmidler, 1971 have similar distributions (Veith et al., 2003; Litvinchuk et al., 2006; Gvoždík, 2010).

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