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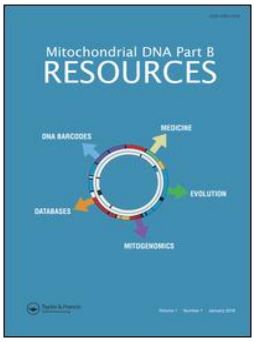

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MITOGENOME ANNOUNCEMENT

The complete mitochondrial genome of a European fire-bellied toad (*Bombina bombina*) from Germany

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ABSTRACT

The European fire-bellied toad, *Bombina bombina*, is a small aquatic toad belonging to the family Bombinatoridae. The species is native to the lowlands of Central and Eastern Europe, where population numbers have been in decline in recent past decades. Here, we present the first complete mitochondrial genome of the endangered European fire-bellied toad from Northern Germany recovered using iterative mapping. Phylogenetic analyses including other representatives of the Bombinatoridae placed our German specimen as sister to a Polish *B. bombina* sequence with high support. This finding is congruent with the postulated Pleistocene history of the species. Our complete mitochondrial genome represents an important resource for further population analysis of the European fire-bellied toad, especially those found within Germany.

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Bombina bombina; Fire-bellied toad; mitogenome; conservation genetics; population delimitation

The European fire-bellied toad (*Bombina bombina*) is a small-bodied toad and one of eight morphologically similar species in the genus *Bombina*. It is widely distributed throughout Eastern and Central Europe (Pabijan et al. 2013; Kuzmin et al. 2008). Despite this widespread distribution, isolated populations at the edge of its distribution range, as in Germany, have been in decline in recent decades due to intensive agriculture and ongoing habitat fragmentation (Arntzen 1978; Dolgener et al. 2012). This led the European fire-bellied toad to be listed as threatened with extinction on the National Red List in Germany (Beutler et al. 1998). Previous studies using complete mitochondrial genomes shed light on the phylogeny of the genus (Pabijan et al. 2013), but did not present individuals from the entire species range, including samples from only three central and southeast European localities (Poland, Turkey, and Austria). Northern German *B. bombina* populations are considered to be genetically depauperated and exhibit local Control Region haplotypes (Schröder et al. 2012), yet, the complete mitochondrial genome of the German *B. bombina* is still not available.

Our sample was a tadpole collected south of Neutestorf in Schleswig-Holstein, Germany (54°23′ N, 10°75′ E) and is permanently stored at the University of Potsdam. DNA and RNA were extracted using a customized Trizol/Chloroform protocol, built into an Illumina sequencing library using a NEXTflex Rapid Directional RNA-Seq Library Prep kit and sequenced on an Illumina HiSeq (Novogene, Hongkong). We trimmed adapter sequences using Cutadapt v.1.4 (Martin 2011) and removed PCR duplicates using Prinseq (Schmieder and Edwards 2011). We used iterative mapping to reconstruct the mitochondrial genome using MITObim v1.9 (Hahn et al. 2013) with default parameters and a mismatch value of 3. Mitochondrial genomes from *B. bombina* from three localities were used as bait sequences for three independent runs, Turkey (Genbank accession JX893172.1), Austria (JX893173.1) and Poland (EU115993.1). We constructed consensus sequences for each independent run using a 65% base call threshold and a minimum read depth of 10× in Geneious v8.1.9 (Kearse et al. 2012). We aligned the resultant consensus sequences using Mafft v7.271 (Katoh and Standley 2013) and called a final consensus sequence using a 75% base call threshold. We automatically annotated the resultant 17,381 bp genome (MH893761) using MITOS (Bernt et al. 2013) and found all protein-coding genes, tRNAs and rRNAs typical for vertebrate mitochondrial genomes. We aligned our sequence with a number of complete mitochondrial genomes from other *Bombina* species from Genbank using ClustalW (Thompson et al. 2002). We then performed a Maximum-Likelihood phylogenetic analysis in RaxML v.8 (Stamatakis 2014) with 500 bootstrap replicates, excluding the control region, and specifying *B.maxima* as outgroup. Results placed our sequence as sister to the Polish specimen with high confidence (bootstrap 100) (Figure 1), a result consistent with the postglacial colonization history, as Poland and Germany is inhabited by the Northern lineage, Austria by the Southern lineage of colonization, and Turkey is close to the assumed glacial refugium (Hofman et al. 2007). This

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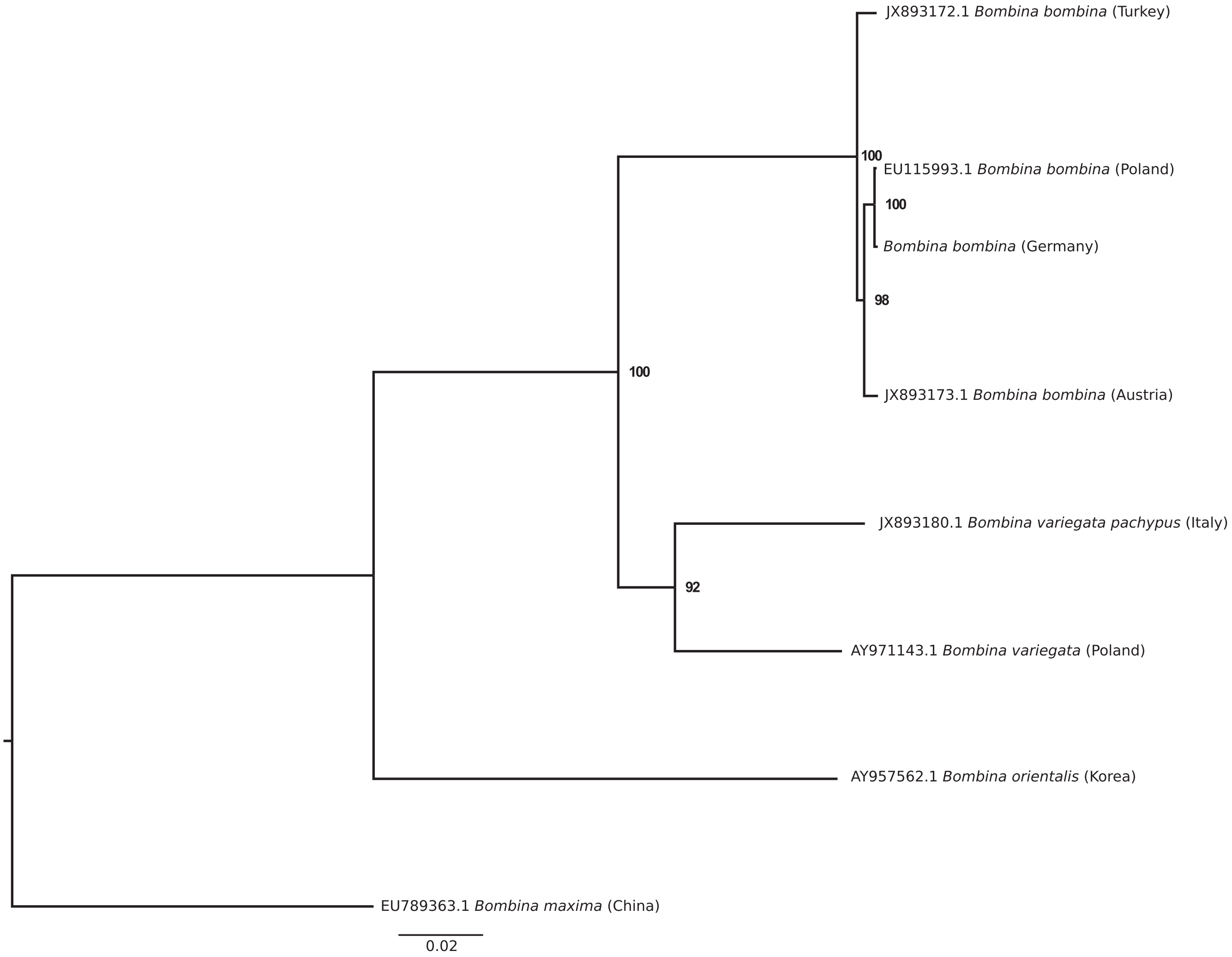

Figure 1. Maximum-Likelihood tree showing the phylogenetic positioning of German *Bombina bombina* in relation to other representatives of the Bombinatoridae family. Numbers on branches represent bootstrap values.

sequence represents an important resource for future studies on German *Bombina bombina* populations.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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