

A revised phylogeny of Alpine newts unravels the evolutionary
distinctiveness of the Bosnian alpine newt – *Ichthyosaura alpestris*
reiseri (Werner, 1902)

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Supplementary material

To identify the position of our samples in the diversity tree of *I. alpestris*, we performed a Bayesian phylogenetic analysis with all *cytb* and *16S* sequences of *I. alpestris* that are available

in public repositories. The entire dataset comprised 105 sequences (29 sequences from this study [1-5 in table 1], 17 sequences from Sotiropoulos et al., 2007 [6-22 in table 1] and 59 other sequences [supplementary table S1]). Sequences were concatenated in Notepad (Microsoft) and aligned in Bioedit v5.09 (Hall 1999) using the ClustalW algorithm (Larkin et al., 2007). The full *cytb*-*16S* alignment was reduced to one representative of each unique haplotype (n = 55) using DnaSp 6.0 (Rozas et al., 2017) under standard settings. To this alignment, we added sequences of *Ommatotriton vittatus* Gray, 1835 (Acc. No.: *cytb* - AY336659 and *16S* - AY336630 from Veith et al., 2004) and *Lissotriton vulgaris* Linnaeus, 1758 (Acc. No.: *cytb* - U55948 and *16S* - U04705 from Caccone et al., 1994) as outgroups.

The resulting alignment (n = 57, 592 bp) was used to reconstruct a phylogenetic tree in Mr Bayes v. 3.1.2 (Ronquist and Huelsenbeck, 2003). First, we conducted a substitution saturation test in DAMBE (Xia and Xie, 2001; Xia et al., 2003) that confirmed that our sequences were suitable for phylogenetic analysis. DAMBE was also used to partition the *cytb* gene (on 1st, 2nd and 3rd codon position); the *16S* gene (non-protein) was not partitioned. JModelTest 2.1.7 (Darriba et al., 2012) showed that for the first partition of *cytb* evolves best under the GTR+G model while the second and third under GTR+I+G model; HKY+G revealed to be the optimal nucleotide substitution model for *16S*. These evolutionary models were chosen according the corrected Akaike Information Criterion (AICc) as suggested by Burnham and Anderson (2004). Bayesian analysis was conducted by specifying the respective evolutionary models for each partition and *16S* sequences under 50,000,000 generations with four parallel chains and sampled every 1000 generations. Two runs were specified but one final tree was generated using a 10% burn-in fraction. The BI tree was visualized and edited with FigTree v. 1.3.1 (Rambaut, 2009).

The phylogenetic tree showed that our samples group with the samples from the E2 subclade of *I. alpestris* (sensu Sotiropoulos et al., 2007) and that are well distributed over it

(supplementary fig. S1 and fig. 1A). Following Sotiropoulos et al. (2007), the E2 subclade, together with its sister subclade E1 (samples from Montenegro), form the E clade that was used to generate the main results of this paper (see main text for reasoning). Other clades retrieved from the phylogenetic analysis are the same as in Sotiropoulos et al. (2007) and match the major retrieved lineages of *I. alpestris* as after Recuero et al. (2014) and Arntzen et al. (2016) (supplementary table S1, supplementary fig. S1 and fig. 1A).

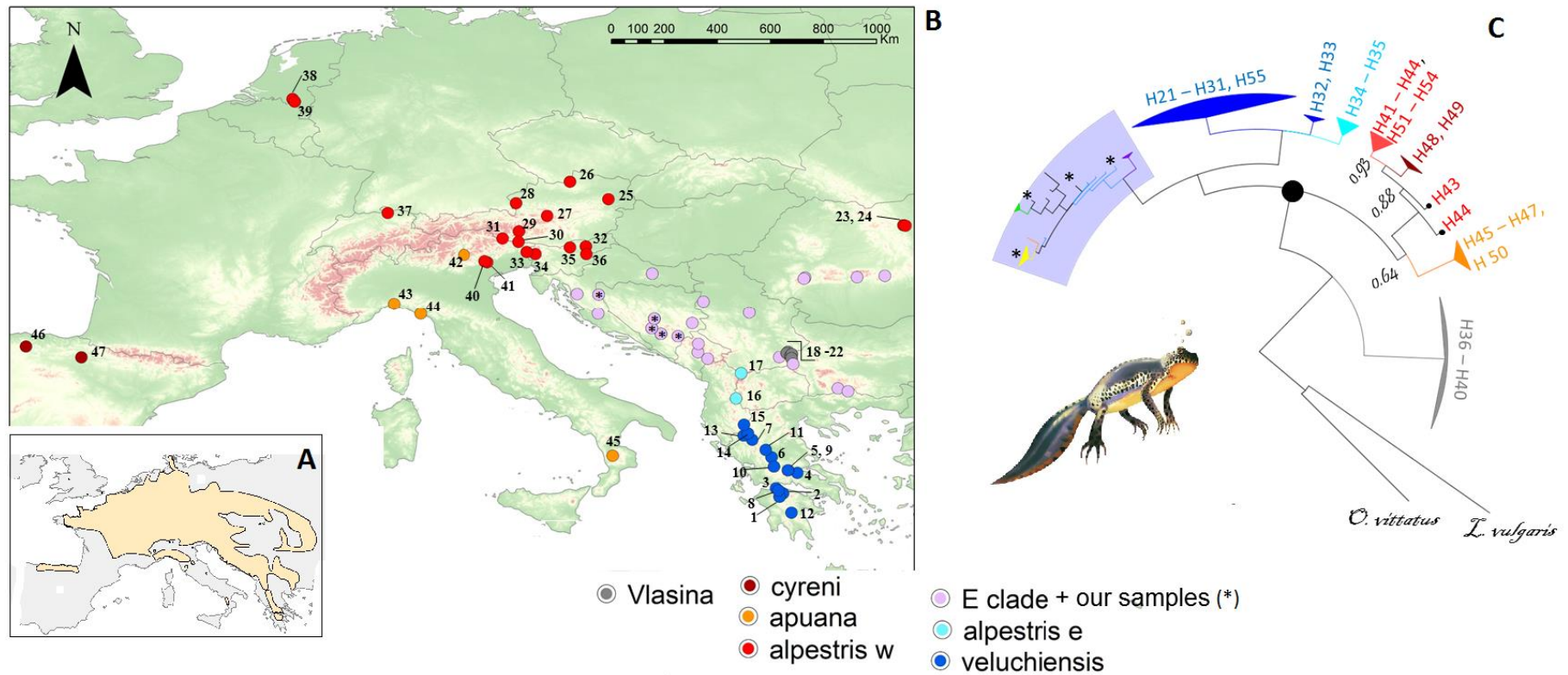


Figure S1. **A** - Distribution of *Ichtyosaura alpestris* (IUCN, 2015); **B** - Map of sampling sites used for the analysis of concatenated *cytb* + 16S sequences of *I. alpestris*; **C** - derived tree of species diversity (mtDNA). Location numbers (codes) and haplotype (H) numbers are as in supplementary table S1. Codes that are not numbered in the map and that are highlighted in the tree (light violet) are samples used to generate the

main results of the paper (our samples + samples from the E clade [sensu Sotiropoulos et al., 2007], see also table 1 and fig. 1); the location of our samples/haplotypes is marked with an asterix (*). The highlighted node in the tree (black circle) shows the split between eastern [e] and western [w] lineages of Alpine newts. Colored dots in the map match the respective clade in the tree (mtDNA based lineages as in the figure legend, following Sotiropoulos et al., 2007; Recuero et al., 2014; Arntzen et al., 2016). Values on tree branches are Bayesian posterior probabilities that are shown only if < 1 . For a simplified representation, the large *veluchiensis* clade in the tree (H21–H31, H55) represents the (intentionally) collapsed haplotypes from the D3 and D4 sister clades (sensu Sotiropoulos et al., 2007). Haplotypes H34 and H35 correspond to the D1 clade (sensu Sotiropoulos et al., 2007, *alpestris* from the eastern lineage – see also supplementary table S1).



Figure S2. Top: Adults of *Ichthyosaura alpestris reiseri* from the captive population maintained in Paris Zoo (A: male; B: female). Bottom: Water bodies around Prokosko Lake explored between the 28th and the 29th of June 2017). Picture C corresponds to the sampling point Wb1 (see fig. 2) and picture D shows a water body around the same area where no newts have been found.

Table S1. Sample localities used in the study. N – latitude; E – longitude; Acc. – accession numbers of sequences in GenBank; Hap – haplotype. Codes for localities and haplotypes are as in supplementary fig. S1. Major lineages of Alpine Newts are derived after Sotiropoulos et al., 2007, Recuero et al., 2014 and Arntzen et al., 2016; in the alpestris lineage, “e” stands for eastern lineage and “w” for western lineage.