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Discordance between phylogenomic methods in Near Eastern mountain newts (*Neurergus*, Salamandridae)

Stephanie Koster [a,b,*], Robin Polanen [a,b], Aziz Avcı [c], Sergé Bogaerts [d], Emin Bozkurt [e], Forough Goudarzi [f], Mahmoud-Reza Hemami [g], Kurtuluş Olgun [c], Frank Pasmans [h], Sebastian Steinfartz [i], Nazan Üzüm [c], Manon de Visser [a,b,j], James France [a,b], Anagnostis Theodoropoulos [a,b], Ben Wielstra [a,b]

[a] Institute of Biology Leiden, Leiden University, P.O. Box 9505 2300 RA Leiden, the Netherlands
[b] Naturalis Biodiversity Center, P.O. Box 9517 2300 RA Leiden, the Netherlands
[c] Department of Biology, Faculty of Sciences, Aydın Adnan Menderes University, 09010 Aydın, Türkiye
[d] Strijperstraat 55a NL5595GB Leende, the Netherlands
[e] Department of Veterinary Medicine, Eldivan Health Services Vocational High School, Çankırı Karatekin University, Çankırı, Türkiye
[f] Environmental Sciences Research Institute, Shahid Beheshti University, G.C, Tehran, Iran
[g] Department of Natural Resources, Isfahan University of Technology, Isfahan, Iran
[h] University of Ghent, Department of Pathobiology, Pharmacology and Zoological Medicine 9820 Merelbeke, Belgium
[i] University of Leipzig, Institute of Biology, Molecular Evolution and Systematics of Animals, Talstrasse 33 04103 Leipzig, Germany
[j] Blijdorp Conservation & Science Center, Royal Rotterdam Zoological & Botanical Gardens, P.O. Box 532 3000 AM Rotterdam, the Netherlands

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ABSTRACT

Target enrichment by sequence capture allows researchers to collect genome-wide sequence data, suitable to re-evaluate complex systematic and taxonomic cases. The family Salamandridae is rife with rapid, successive speciation events, introgression and incomplete lineage sorting: factors that complicate the inferring of phylogenetic relationships and species boundaries. Phylogenetic relationships among, and the (sub)species status of, the taxa comprising the newt genus *Neurergus* are not fully resolved. We perform target enrichment by sequence capture to obtain ca. 7 k nuclear DNA markers, and conduct concatenated analysis with RAxML, gene-tree summary analysis in ASTRAL, and species tree estimation in SNAPPER, and investigate admixture between populations and genetic introgression with ADMIXTURE and Dsuite. We observe discordance between analyses pertaining to the placement of *N. crocatus*. We consider the placement of *N. crocatus* as sister to *N. derjugini* sensu lato in SNAPPER, instead of to *N. kaiseri* + *N. derjugini* sensu lato as in RAxML and ASTRAL, to be an artifact of introgression, as backed up by our Dsuite analysis. We show that *N. strauchii barani* and *N. strauchii munzurensis*, often treated as distinct species, are deeply nested within *N. strauchii* sensu stricto and should not be treated as species. Keeping them as subspecies would render the nominotypical subspecies as polyphyletic. Furthermore, we confirm that *N. derjugini microspilotus* should not be considered a distinct species, but as a subspecies of *N. derjugini* sensu lato. We consistently recover the northern and southern lineages of *N. kaiseri* as distinct genetic groups with geographically restricted admixture and recommend that these are treated as two distinct species. Our study highlights the strength of target enrichment by sequence capture in resolving systematic and taxonomic questions in taxa with a history of genetic admixture and introgression.

1. Introduction

Genome-wide data have provided a boost for systematics and taxonomy, allowing researchers to disentangle evolutionary relationships all over the tree of life (Ekblom & Galindo, 2011; McCormack et al., 2013). However, there are many factors that complicate these efforts. This is especially true for speciation events that occurred in quick succession, resulting in a weak phylogenetic signal due to short internal branches and incomplete lineage sorting (Degnan & Rosenberg, 2009). Furthermore, introgression during and after speciation can obscure the

* Corresponding author at: Institute of Biology Leiden, Leiden University, P.O. Box 9517 2300 RA, Leiden, The Netherlands.
E-mail address: sca.koster@outlook.com (S. Koster).

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true branching order (Kutschera et al., 2014). As a result a large proportion of individual gene trees may be incongruent with the actual species tree (Gee, 2003; Lozano-Fernandez, 2022).

Target enrichment by sequence capture is a way to obtain standardized data across the genome for relatively genetically diverged taxa, without the need for whole genome sequencing (Andermann et al., 2020). This approach helps to clarify systematics and taxonomy, especially in situations where genome-scale data are necessary to help resolve evolutionary questions, but whole-genome sequencing may not be feasible due to e.g. large genome size or limited resources (Andermann et al., 2020; McCartney-Melstad et al., 2016). Standardized protocols are being developed for a wide range of taxa; e.g. NewtCap (De Visser et al., 2024a) can be applied to the entire salamander family Salamandridae (Amphibia: Caudata).

In Salamandridae, the family that encompasses the true salamanders and newts, quick, successive speciation events that affect the inference of relationships between and within genera are prevalent (Rancilhac et al., 2021; Weisrock et al., 2006; Zhang et al., 2008). Furthermore, this family shows extensive evidence of both recent and historical gene flow (De Visser et al., 2024b; Pabijan et al., 2017; Rancilhac et al., 2021; Stuglik and Babik, 2016; Wielstra et al., 2019), which partly explains why taxonomic assignments based on single markers, typically mtDNA, have later been proven wrong with genome-scale data (Kalaentzis et al., 2025; Mars et al., 2025; Rancilhac et al., 2019, 2021).

One case that could benefit from a phylogenomic investigation is *Neurergus*, a genus referred to as the Near Eastern brook newts, that occurs in the Irano-Anatolian biodiversity hotspot (Khoshnamvand et al., 2024). Although most of the taxa within *Neurergus* are considered to be vulnerable to extinction by the IUCN as a result of habitat loss and degradation, as well as overharvesting for the pet trade (IUCN SSC Amphibian Specialist Group, 2016, 2021, 2023a, 2023b, 2023c), studies on this genus have so far focused on only a few genetic markers and/or populations. Questions remain regarding the systematics of *Neurergus*, particularly regarding the phylogenetic position of *N. crocatus* (Khoshnamvand et al., 2024; Rancilhac et al., 2019; Vaissi, 2021).

The taxonomy of *Neurergus* is also not fully settled. The status of the three taxa comprising *N. strauchii* sensu lato, *N. s. strauchii*, *N. s. barani* and *N. s. munzurensis*, is unclear. Previous studies disagree on whether *N. s. strauchii* and *N. s. barani* should be treated as distinct species (Hendrix et al., 2014; Özdemir et al., 2009; Rancilhac et al., 2019; Steinfartz et al., 2002). The recently described *N. s. munzurensis* has never been studied from a genomic perspective (Olgun et al., 2016), but has been suggested to represent a distinct species (Frost, 2024). Furthermore, it is known that there are two morphologically and genetically distinct lineages within *N. kaiseri*, a northern and a southern one, but no clear taxonomic recommendations have been proposed (Farasat et al., 2016; Goudarzi et al., 2019; Khoshnamvand et al., 2018, 2019; Rancilhac et al., 2019; Vaissi, 2021; Vaissi & Sharifi, 2021). Lastly, *N. derjugini microspilotus* has been treated as a distinct species or as a subspecies of *N. derjugini* sensu lato (Hendrix et al., 2014; Rancilhac et al., 2019; Schneider & Schneider,

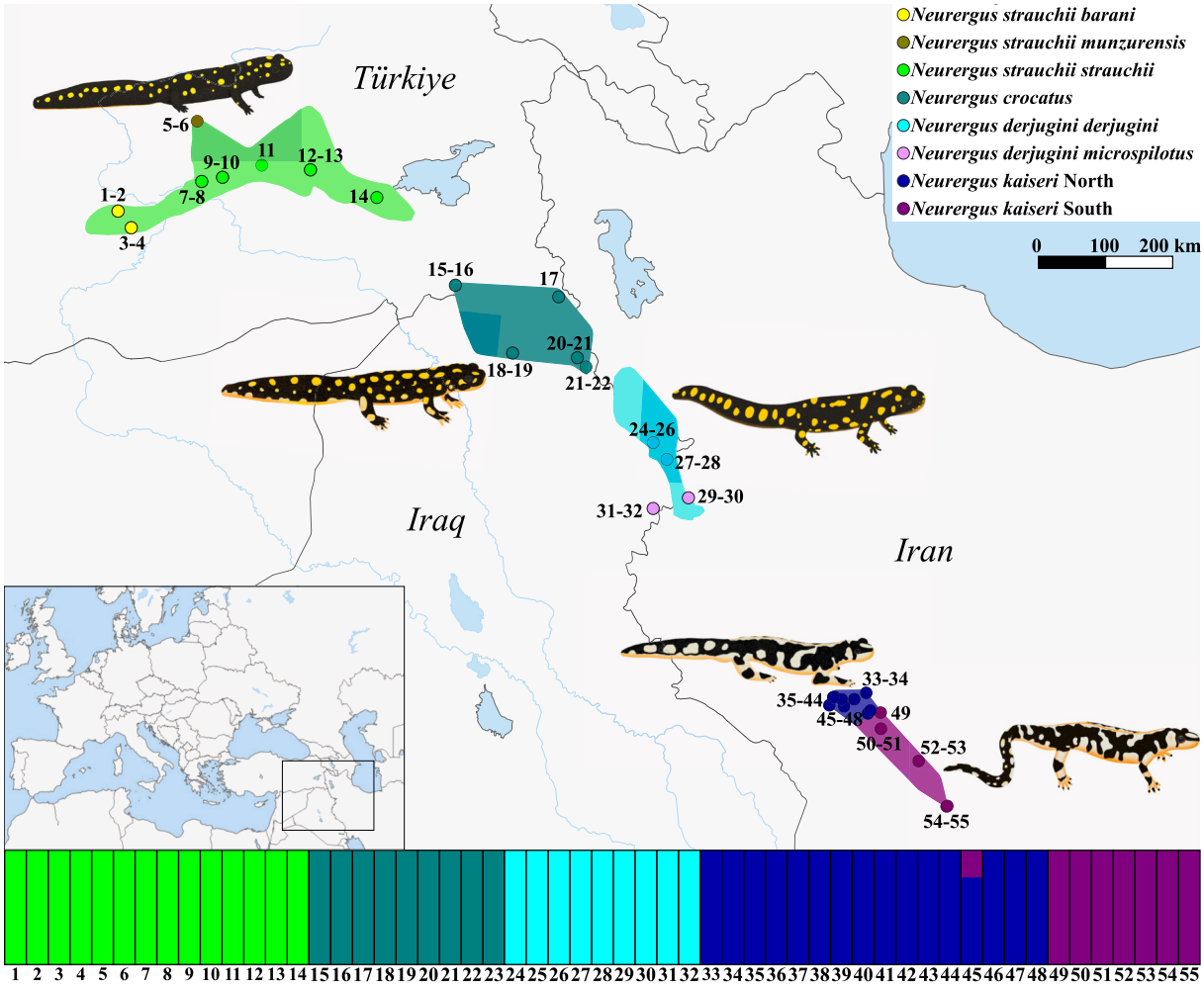

Fig. 1. Distribution map with admixture proportions for the Near Eastern mountain newts (genus *Neurergus*). The polygons show a rough outline of the distribution of the five species proposed in the present study, from top to bottom; *N. strauchii*, *N. crocatus*, *N. derjugini*, and the as yet unnamed northern and southern lineage of *N. kaiseri*. The color of sampled populations corresponds to (sub)species. Sample numbers correspond to Table S1.

2013).

Our aim is to resolve the systematics and taxonomy of the genus *Neurergus*. We combine genome-wide data collection through target enrichment by sequence capture with high-resolution geographical sampling of all known taxa to infer the phylogenetic history of *Neurergus* and investigate hybridization and introgression. Our comprehensive approach allows us to revise the taxonomy and systematics of *Neurergus* newts.

2. Materials & methods

2.1. Sampling

We included 55 *Neurergus* samples available from previous studies (Goudarzi et al., 2019; Olgun et al., 2016; Pasmans et al., 2006). These samples cover the natural range of *Neurergus* (Fig. 1), and for most populations more than one individual was sampled (Table S1). For outgroup comparison, we included the closely related Salamandridae taxa *Triturus carnifex*, *T. macedonicus* and *T. marmoratus* with three individuals per species (data taken from Kazilas et al., 2024; Wielstra et al., 2019).

2.2. Library preparation and sequence capture

We used the NewtCap sequence capture protocol (De Visser et al., 2024a) to obtain genomic data. DNA extraction was performed with the Promega Wizard™ Genomic DNA Purification kit (Promega, Madison, WI, USA), and we then conducted library preparation with the NEBNext® Ultra™ II FS DNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA, USA). A Fragment Analyzer system (Agilent, Santa Clara, CA, USA) was used to assess library concentration and quality. We then performed target enrichment with a custom probe set based on *Triturus* transcriptome data that targets 7,139 exonic regions (MyBaits v4.0 kit, Arbor Biosciences Ref# 170210–32; Wielstra et al., 2019). Before the pools were sent to BaseClear B.V. (Leiden, the Netherlands) for 150 bp paired-end sequencing on the NovaSeq 6000 platform (Illumina Inc., San Diego, CA, USA, aiming for 1 Gb of data per sample, their concentration and size distribution were analyzed on the Agilent 220 TapeStation system (Agilent, Santa Clara, CA, USA).

2.3. Bioinformatics

An upstream bioinformatics pipeline described in the NewtCap sequence capture protocol (De Visser et al., 2024a) was used to check the quality of the data, and to clean, map and combine the reads. Trimmed reads were mapped to a reference sequence consisting of a *Triturus* reciprocal best blast hit (RBBH) contig assembly reference with 6,884 targets (Wielstra et al., 2019). For the variants in the multi-sample VCF resulting from the pipeline, Hardy Weinberg-related statistics were calculated with BCFtools v.1.15.1 (Danecek et al., 2021), and any variant sites with heterozygote excess ($p < 0.05$) were filtered out from the dataset. Insertions/deletions and sites with a genotype quality below 20 or with missing data in more than 50 % of samples were also filtered out using VCFtools v.0.1.16 (Danecek et al., 2011). This filtered set was used for the RAxML analysis, whereas for the ADMIXTURE, ASTRAL and SNAPPER analyses all sites with missing data were removed. A custom R script was used to determine the coverage of each sample (France et al., 2024).

2.4. Admixture analysis

An admixture analysis for the full *Neurergus* dataset (55 samples) was performed in ADMIXTURE v1.3.0 (Alexander et al., 2009), to investigate whether any of the samples showed evidence of genetic admixture that could interfere with downstream analyses. First, one SNP was randomly selected for each of the 5,168 markers using a custom Perl script. PLINK v1.07 (Purcell et al., 2007) was then used to create the input file, and ADMIXTURE was run for number of ancestral populations K 1–20, with 25 iterations each, to determine the optimal number of ancestral populations via cross validation. ADMIXTURE was performed again for this optimal number, and the results were summarized using CLUMPAK (Kopelman et al., 2015). The resulting output file was plotted in R (R Core Team, 2024; RStudio Team, 2024; Sethuraman, 2014).

2.5. RAxML

Three *Triturus* species were added to the *Neurergus* dataset as outgroup. Based on ADMIXTURE (see Results), one sample showing admixture between the northern and southern lineages of *N. kaiseri* (BW_1950; Table S1) was excluded. This resulted in a total of 63 samples. Sites with heterozygote excess and over 50 % missing data were removed from the VCF file. This file was then converted into PHYLIP format using the vcf2phy.py Python script (Ortiz, 2019). Any sites that would be considered invariable by RAxML were removed from the resulting PHYLIP file with the Python script ascbias.py (https://github.com/btmartin721/raxml_ascbias). A concatenated maximum likelihood phylogenetic tree was then inferred in RAxML v.8.2.12 (Stamatakis, 2014), based on 171,092 SNPs across 6,884 targets. We conducted one hundred rapid bootstrap replicates with the ASC_GTRGAMMA model with Lewis ascertainment correction for SNP analysis (Leaché et al., 2015; Lewis, 2001; Wang & Nielsen, 2012).

The phylogeny was rooted on the branch between *Triturus* and *Neurergus* in FigTree v1.4.4 (https://tree.bio.ed.ac.uk/software/figtree/), and used as input for a dated inference in treePL v2.6.3 (Smith & O'Meara, 2012). We used two fixed calibration points; the split between *T. carnifex* and *T. macedonicus*, dated to 5.33 Ma, and the basal split between *T. marmoratus* and the former, dated to 24 Ma (Steinfartz et al., 2007; Wielstra & Arntzen, 2011). A priming analysis was performed in treePL to obtain optimal parameters to be used in the final analysis (Maurin, 2020). The optimization parameters used were as follows; opt = 2, moredetail, optad = 3, moredetailad and optcvad = 5. The optimal smoothing value was 0.0000000001. To add 95 % confidence intervals to the phylogeny, we ran 100 bootstrap replicates using RAxML. Topologies were fixed, but the branch lengths were not. The treePL analysis was then repeated for each replicate, and the trees were summarized in TreeAnnotator v2.4.7 (Bouckaert et al., 2019).

2.6. ASTRAL

For ASTRAL v5.7.8 (Zhang et al., 2018), we split our 63 sample VCF file (with *Triturus* outgroup, excluding the admixed sample BW_1950; Table S1), with all sites that had missing data removed, into separate files for each target marker using SnpSift v.4.3 (Cingolani et al., 2012). The separate VCF files were converted to PHYLIP format with PGDspider 2.1.1.5 (Lischer & Excoffier, 2012), and invariable sites were removed as above. We then used RAxML to infer gene trees for the separated targets, with the ASC_GTRGAMMA model and Lewis ascertainment bias correction. A total of 4,315 gene trees were concatenated in a single file, and used as input for ASTRAL. Guided by ADMIXTURE (see Results), samples belonging to *N. s. barani* and *N. s. munzurensis* were included under *N. strauchii* sensu lato, the samples for *N. d. microspilotus* were included under *N. derjugini* sensu lato, and the northern and southern lineages of *N. kaiseri* were treated as two separate genetic groups.

2.7. SNAPPER

For SNAPPER v1.1.3 (Stoltz et al., 2021), a VCF file with one randomly selected SNP each for 5,168 markers was generated from the 54 sample VCF file (without admixed sample BW_1950; Table S1) with a custom Perl script. This file was then converted into binary NEXUS format using the Python script vcf2phylip.py (https://github.com/edgardomortiz/vcf2phylip), resulting in a file with 4,974 biallelic SNPs. The

binary NEXUS file was converted to the XML format in BEAUti v.2.7.5 in BEAST v2.7.4 (Bouckaert et al., 2019), with default parameters and a chain length of 15,000,000. Guided by ADMIXTURE (see Results), samples belonging to *N. s. barani* and *N. s. munzurensis* were included under *N. strauchii* sensu lato, samples for *N. d. microspilotus* were included under *N. derjugini* sensu lato, and the northern and southern lineages of *N. kaiseri* were treated as two separate genetic groups. SNAPPER was run within BEAST. We removed 10 % burn-in from the output file with TreeAnnotator v.2.7.5 (Bouckaert et al., 2019), and all remaining phylogenies were combined with the default settings. Since the topology of SNAPPER might be affected by the inclusion of an outgroup (Kalaentzis et al., 2025), we performed a second run on the 63 sample VCF file (i.e. with *Triturus* outgroup included) with 5,325 targets. The binary NEXUS file for this set contained 5,013 biallelic SNPs. Due to computational limitations, we performed six runs, each with a chain length of 5,000,000. Otherwise, the same settings as before were used for SNAPPER. We combined the runs using LogCombiner v2.7.5 (Bouckaert et al., 2019), after checking if they converged on the same result in Tracer v1.7.2 (Rambaut et al., 2018) and removing 10 % burn-in from each. Tracer was also used to determine the final ESS values. The resulting trees were then visualized in FigTree v.1.4.4 (https://tree.bio.ed.ac.uk/software/figtree/).

2.8. Introgression analyses with Dsuite

The unfiltered VCF file, containing 682,428 biallelic SNPs, was used for Dsuite v0.5-r48 (Malinsky et al., 2021). The Dtrios function was used to calculate the *f4* admixture ratio statistic for all possible *Neurergus* species trios, with *Triturus* as outgroup. Then, we used the Fbranch function to determine *f*-branch statistics for all positive *f4* admixture ratios, based on the ASTRAL phylogeny for *Neurergus*. This approach can untangle admixture signals by time periods, and assign those to (potentially internal) branches on the tree (Malinsky et al., 2021). The dtools.py Python script included with Dsuite was used to visualize the results as a heatmap.

2.9. MtDNA analysis

We sequenced 674 bp of the mtDNA gene ND4 (NADH dehydrogenase subunit 4). In Geneious Prime 2021.1.1 we adjusted the 'universal' ND4 primers (ND4 and Leu) from Arévalo et al. (1994) to match the sequence in the mitogenomes of *N. strauchii* (GenBank accession EU880321) and *N. kaiseri* (EU880320) published by Zhang et al. (2008), taking degenerate sites into account. This resulted in the primers ND4_Neu (5′-CAYTTATGRTTACCAAAAGCCCAYGTAGAAGC-3′) and Leu_Neu (5′- CATAGCTTTTACTYGGARTTGCACCA-3′).

PCRs were performed in 12 µl reactions, containing 0.06 µl of both forward and reverse primer (0.05 µM end concentration of each primer), 7.2 µl QIAGEN multiplex PCR master mix, 3.68 µl purified water and 1 µl of DNA extract. PCR conditions were: a hot start for 15 min at 95 °C, followed by 35 cycles of denaturation for 30 s at 95 °C, annealing for 1 min at 55 °C and extension for 1 min at 72 °C, and final extension at 72 °C of ten minutes. Sanger sequencing was outsourced to BaseClear B. V. Sequences were edited and trimmed in Geneious Prime 2023.1.1 and the DNAcollapser function in FaBox (Villesen, 2007) was used to collapse sequences into haplotypes.

We inferred a Maximum Likelihood tree with RAxML v8.2.12 (Stamatakis, 2014), based on 231 distinct alignment patterns. A partition file was passed to RAxML, so that model parameters would be estimated and optimized for each codon position. We conducted 100 bootstrap replicates with the GTRGAMMA model. FigTree v.1.4.4 (https://tree.bio.ed.ac.uk/software/figtree/) was used for rooting and visualization.

3. Results

The 48 Gb of raw sequence capture data obtained contains on average 12,567,736 (s.d. 5,682.527) read pairs per sample. Of the filtered reads, on average 33.5 % (s.d. 5.1 %) map to our reference. The mean percentage of duplicate reads is 80.5 % (s.d. 7.9 %). The average number of targets that has at least one read is 6,834 (s.d. 52.8), and the median peak 100 bp coverage of the samples after de-duplication is 30.5 (s.d. 29.3) on average, ranging from 8 to 159.

The optimal number of ancestral populations for the ADMIXTURE analysis is five. We see *N. strauchii* sensu lato recovered as a single group that includes the nominotypical subspecies as well as *N. s. munzurensis* and *N. s. barani*. The northern and southern lineages of *N. kaiseri* are recovered as two separate genetic groups (Fig. 1). One sample from the northern lineage (BW_1950) shows evidence of extensive admixture with the southern lineage and is therefore not included in downstream analyses. *Neurergus derjugini* sensu lato forms a single genetic group, and so does *N. crocatus*.

The concatenated RAxML analysis (Fig. 2) suggests that *N. s. barani, N. s. munzurensis* and *N. s. strauchii* are not reciprocally monophyletic. We recover *N. d. derjugini* and *N. d. microspilotus* as reciprocally monophyletic, closely related lineages. The samples of *N. crocatus* are recovered as a monophyletic group. The northern and southern lineages of *N. kaiseri* are recovered as reciprocally monophyletic groups. Repeating the RAxML analysis with the admixed sample (BW_1950) included shows it clusters with the northern *N. kaiseri* lineage, but is pulled to a basal position (Fig. S1). The basal split separates *N. strauchii* sensu lato from the other *Neurergus* taxa. The next taxon to split off is *N. crocatus*. Finally, *N. derjugini* sensu lato is recovered as the sister lineage to *N. kaiseri*. Crown ages are ca. 12.3 Ma for *Neurergus*, ca 7.0 Ma for *N. derjugini* sensu lato + *N. kaiseri*, ca. 3.4 Ma for *N. strauchii* sensu lato, ca. 3.2 Ma for *N. kaiseri*, ca. 2.6 Ma for *N. crocatus*, and ca. 1.3 Ma for *N. derjugini* sensu lato.

In ASTRAL we use the five genetic groups as indicated by the ADMIXTURE analysis, further supported by the concatenated RAxML analysis, as 'species'. The gene tree summary analysis with ASTRAL (Fig. 3) recovers the same topology as the concatenated RAxML (Fig. 2) analysis. All nodes receive full support, except for the split between *N. kaiseri* and *N. derjugini* sensu lato, which receives a posterior probability of 0.96. The normalized quartet score, representing the proportion of quartet trees generated by all gene trees that is present in the species tree, and as such is a measure for gene-tree discordance, is 0.62.

In SNAPPER we again use the five genetic groups as indicated by the ADMIXTURE and RAxML analysis as 'species'. The SNAPPER analysis (Fig. 3) recovers a different topology from RAxML (Fig. 2) and ASTRAL (Fig. 3). Now *N. crocatus* is recovered as the sister lineage of *N. derjugini* sensu lato. This topology is consistent between runs with and without an outgroup included (Fig. S2), and nodes receive full support. ESS values were high (>200) for both runs.

The Dsuite analysis (Fig. 4) signals several instances of introgression within *Neurergus*, particularly between *N. derjugini* sensu lato and *N. crocatus*.

For 53 individuals we obtained the targeted 674 bp of ND4. These contain 29 haplotypes. For the two remaining individuals (BW_2163 and BW_2164) we obtained a 332 bp sequence, which was identical in both individuals. Our mtDNA RAxML analysis (Fig. 5) suggests that *N. s. barani, N. s. munzurensis* and *N. s. strauchii* are not reciprocally monophyletic. The same applies to *N. d. derjugini* and *N. d. microspilotus*. All other groups, i.e. *N. crocatus* and the northern and southern lineages of *N. kaiseri* were recovered as monophyletic. While the basal split is between *N. strauchii* sensu lato and the rest, relationships among the remaining taxa are not resolved.

4. Discussion

We investigate the phylogenetic relationships of the entire newt

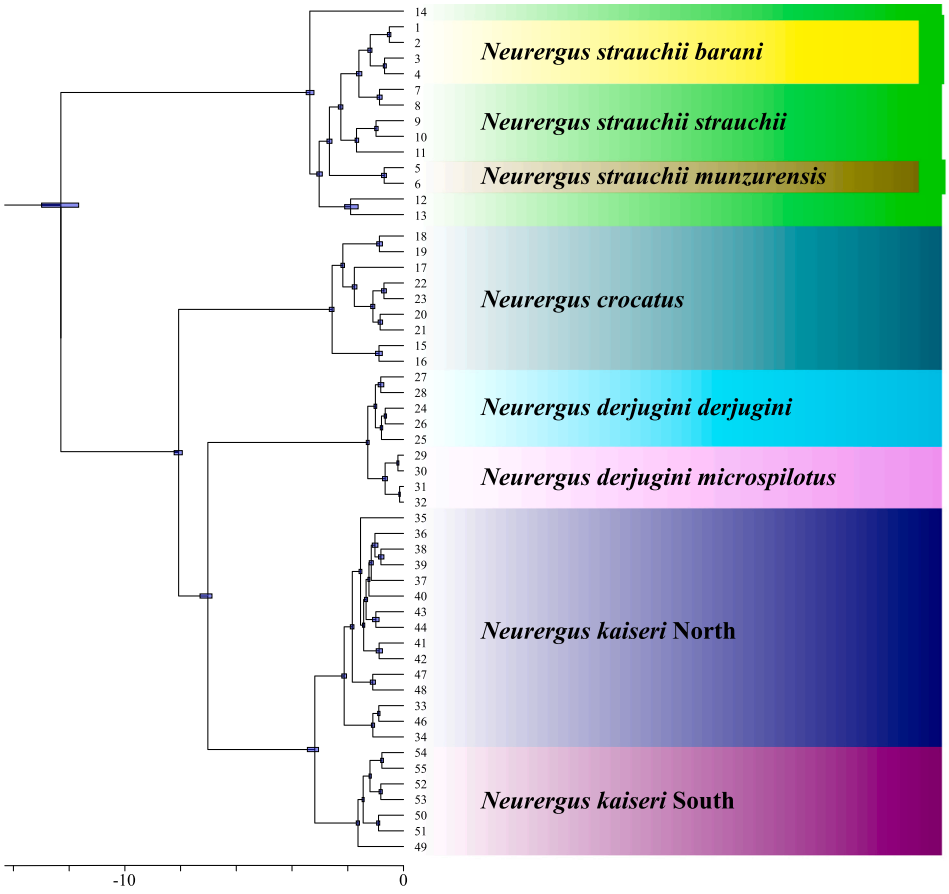

Fig. 2. Time-calibrated phylogeny of the Near Eastern mountain newts (genus *Neurergus*). Time calibration was performed in treePL using a maximum likelihood inference based on data concatenation in RAxML. The 95% confidence intervals are based on 100 bootstrapped RAxML trees, which had their topology but not their branch lengths fixed. Labels correspond to Table S1. The scale bar reflects millions of years ago. The outgroup is not shown.

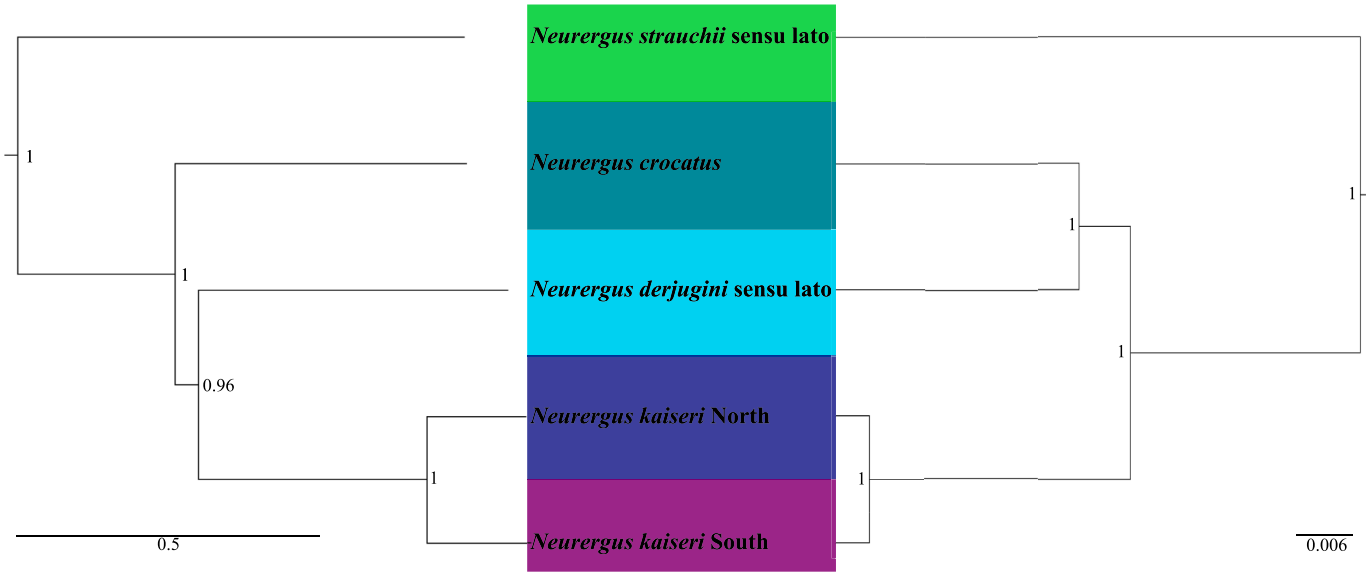

Fig. 3. Multi-species coalescent analysis phylogeny inferred in ASTRAL and Bayesian species-tree inference analysis using a diffusion model in SNAPPER for the Near Eastern mountain newts (genus *Neurergus*). The outgroup is not shown. The branch lengths for ASTRAL represent coalescent units and the numbers on the nodes local quartet support posterior probabilities. The branch lengths for SNAPPER represent the number of expected substitutions per site and node values represent posterior probabilities. This SNAPPER analysis did not include an outgroup, see Fig. S2 for the analysis with outgroup.

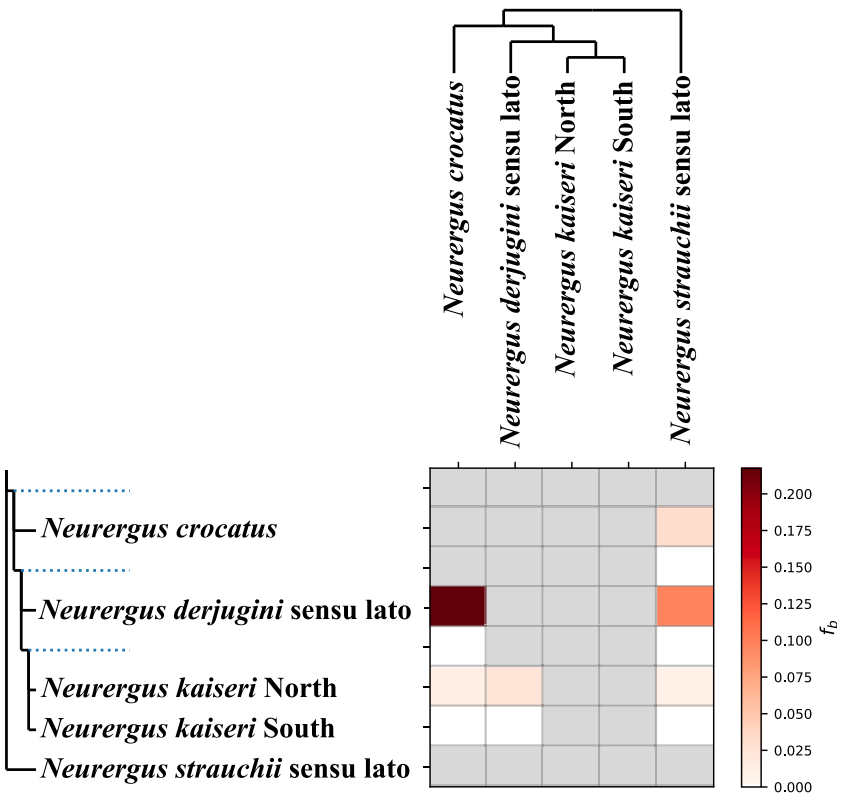

Fig. 4. Dsuite f-branch (fb) heatmap for the Near Eastern mountain newts (genus *Neurergus*). The color shading is a measure for the intensity of excess allele-sharing (i.e. introgression) between tree branches on the y-and x-axes. No f-branch statistics could be calculated for the gray cells. The dotted blue lines correspond to the internal branches of the tree. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

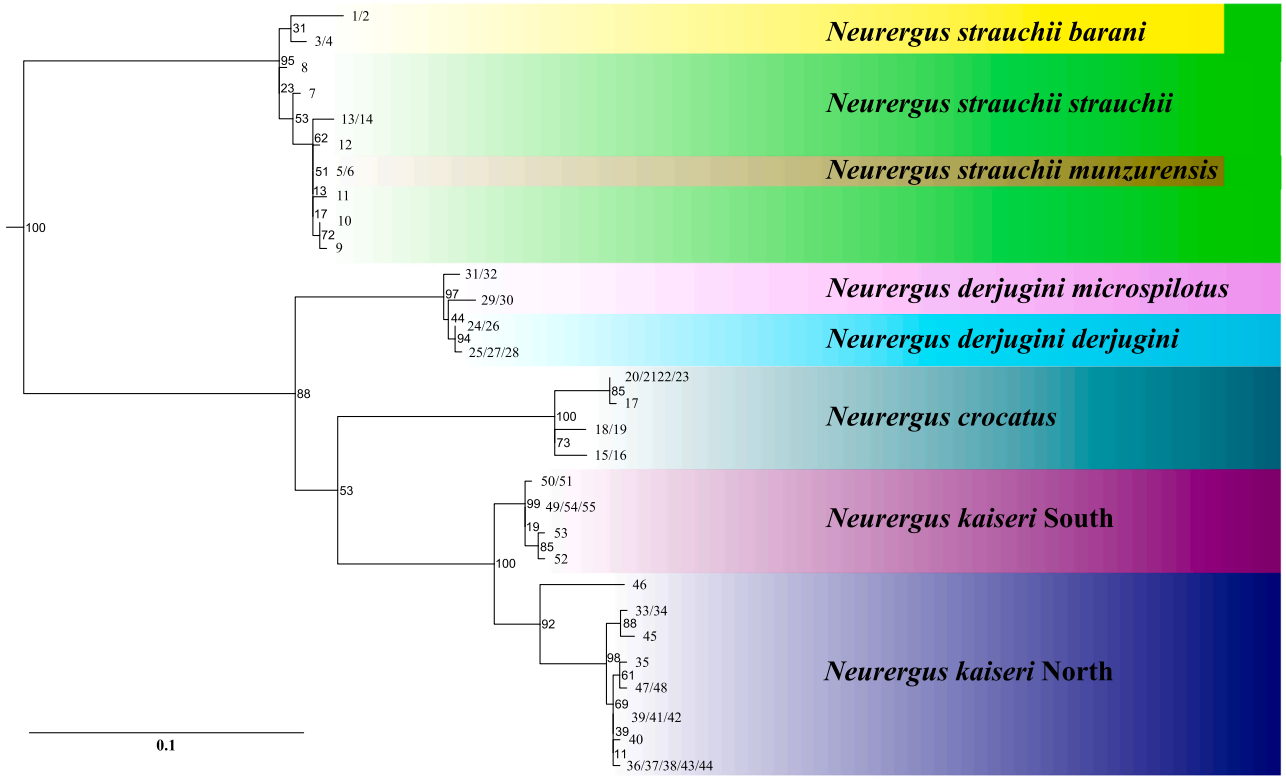

Fig. 5. MtDNA phylogeny of the Near Eastern mountain newts (genus *Neurergus*), based on maximum likelihood inference of concatenated data with RAxML. The node values represent bootstrap support values. The scale bar represents the number of expected substitutions per site.

genus *Neurergus*, using phylogenomics on a dataset obtained through target enrichment by sequence capture. Our phylogenetic analyses generally infer identical topologies, with one notable exception discussed below. Our new insights allow us to make several taxonomic recommendations.

4.1. Introgression complicates phylogenetic inference

There is disagreement among our phylogenetic analyses regarding the position of *N. crocatus*. Whilst RAxML (Fig. 2) and ASTRAL (Fig. 3) suggest a sister relationship between *N. derjugini* sensu lato and *N. kaiseri*, with *N. crocatus* taking a basal position, SNAPPER (Fig. 3) suggests *N. crocatus* is the sister taxon to *N. derjugini* sensu lato while *N. kaiseri* takes a basal position. The same conflict between phylogenetic methods was independently found in a RAD sequencing-based study on *Neurergus* (Rancilhac et al., 2019). Although our ASTRAL topology agrees with RAxML, the normalized quartet score was 0.62, suggesting a substantial amount of gene tree discordance. Our Dsuite analysis (Fig. 4) reveals extensive introgression between *N. derjugini* sensu lato and *N. crocatus*, suggesting this may be responsible for the discordant placement of *N. crocatus* in SNAPPER. Our mtDNA analysis does not manage to resolve relationships among species, as in previous attempts based on less complete sampling (Hendrix et al., 2014; Steinfartz et al., 2002).

4.2. Historical biogeography

Caution has been urged when basing molecular dating solely on SNP data (Leaché et al., 2015). While SNP-based analyses increase computational efficiency, they may compromise the accuracy of branch length estimates—particularly when missing data are substantial (Leaché et al., 2015). However, given the low proportion of missing data in our dataset and the similarity of our calibration times to those used in previous studies (Goudarzi et al., 2019; Khoshnamvand et al., 2024; Vaissi, 2021; Steinfartz et al., 2002), we present insights from a SNP-only analysis here. Divergence times in *Neurergus* match up with geological and climatological changes in the area. The Zagros mountains, which separate *N. strauchii* sensu lato from the rest of the genus, started rising up around 15 Ma (Khoshnamvand et al., 2024). Subsequent humid climate conditions likely exacerbated the spread of *Neurergus* to the south-east, and the formation of mountain valleys led to populations becoming isolated (Vaissi 2021). The formation of the Dez river, which acts as the boundary between the two lineages of *N. kaiseri*, occurred roughly 3–3.5 Ma (Goudarzi et al., 2019). The Zagros mountains presumably also acted as a glacial refugium during the Quaternary Ice Age starting 2.5 Ma (Vaissi, 2021; Steinfartz et al., 2002).

4.3. Taxonomic recommendation to treat several taxa as subspecies instead of species

Our concatenated RAxML analysis based on genome-wide nuclear DNA data (Fig. 2) shows that *N. s. barani* and *N. s. munzurensis* are deeply nested within *N. s. strauchii*. Our ADMIXTURE (Fig. 1) analysis does not recover these as separate genetic entities, and our mtDNA analysis does not support reciprocal monophyly either (Fig. 5). In previous studies based on mtDNA and nuclear DNA high genetic divergence was found between *N. s. barani* and *N. s. strauchii* (Hendrix et al., 2014; Özdemir et al., 2009; Rancilhac et al., 2019), which has led some authors to propose their treatment as distinct species. However, these studies suffered from incomplete sampling. Furthermore, here we are presenting the first genomic data for *N. s. munzurensis*. Based on our analyses, we recommend that *N. s. barani* and *N. s. munzurensis* should not be treated as distinct species. However, we note that if they were to be retained as subspecies of *N. strauchii* sensu lato that would render the nominotypical subspecies polyphyletic. We agree with e.g. Dufresnes et al. (2023) that subspecies should reflect monophyletic groups and suggest *N. s. barani* and *N. s. munzurensis* be synonymized with *N. strauchii*.

Our concatenated RAxML analysis (Fig. 2) recovers *N. d. derjugini* and *N. d. microspilotus* as reciprocally monophyletic groups, albeit closely related ones. They are not recovered as distinct groups in our ADMIXTURE analysis (Fig. 1). Our mtDNA analysis also does not recover them as reciprocally monophyletic (Fig. 5). While the two taxa have a turbulent taxonomic history and have been treated as two distinct species in the past (Fleck, 2010; Nader, 1969; Nikolskii, 1918; Schmidtler & Schmidtler, 1970, 1975; Schneider & Schneider, 2011, 2013), our findings support the recent view to consider *N. d. microspilotus* as a subspecies of *N. derjugini* (Hendrix et al., 2014).

4.4. Taxonomic recommendation to split a taxon into two species

All of our analyses suggest that *N. kaiseri* comprises two genetically distinct groups. ADMIXTURE partitions our samples into a northern and a southern lineage and reveals only a single instance of genetic admixture where the two meet (sample 45, Fig. 1; sample 46 from the same population belongs to the northern lineage). The distance between the closest *N. kaiseri* individuals belonging to the northern and a southern lineage is c. 15 km (samples 46–48 and 49). Our RAxML analyses based on either genome-wide nuclear DNA or mtDNA data recover them as reciprocally monophyletic lineages (Figs. 2 and 5).

The genetic distinctness and limited genetic admixture between these lineages was already reported in previous studies (Goudarzi et al., 2019; Khoshnamvand et al., 2018, 2019; Rancilhac et al., 2019; Vaissi, 2021). These two lineage also express morphological and ecological niche differences (Goudarzi et al., 2021; Khoshnamvand et al., 2018). Our study emphasizes that the genetic turnover between the northern and a southern lineage occurs over a short geographical distance while, evidently, they have the opportunity to hybridize in nature: direct evidence for genetic isolation (Dufresnes et al., 2023; Kalaentzis et al., 2023; Vences et al., 2024).We recommend that the northern and southern lineages of *N. kaiseri* should be treated as two distinct species. The type locality for *N. kaiseri* is "Shahbazan, Luristan, Iran" (Frost, 2024; Schmidt, 1955), which is in the range of the northern lineage. Therefore, the southern lineage should be described as a new species.

5. Conclusions

Our study shows that target enrichment by sequence capture is a powerful approach to obtain genome-wide sequence data with high phylogenetic resolution that can be used to tackle systematic and taxonomic questions. We provide a phylogenomic hypothesis for the newt genus *Neurergus* and highlight a discordance between methods that we ascribe to introgression. Our analyses allow us to make taxonomic recommendations involving both splitting and lumping of species.

CRediT authorship contribution statement

Stephanie Koster: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Robin Polanen:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Aziz Avcı:** Writing – review & editing, Resources. **Sergé Bogaerts:** Writing – review & editing, Resources. **Emin Bozkurt:** Writing – review & editing, Resources. **Forough Goudarzi:** Writing – review & editing, Resources. **Mahmoud-Reza Hemami:** Writing – review & editing, Resources. **Kurtuluş Olgun:** Writing – review & editing, Resources. **Frank Pasmans:** Writing – review & editing, Resources. **Sebastian Steinfartz:** Writing – review & editing, Resources. **Nazan Üzüm:** Writing – review & editing, Resources. **Manon de Visser:** Writing – review & editing, Methodology, Investigation, Formal analysis. **James France:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Anagnostis Theodoropoulos:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Ben Wielstra:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2025.108386.

Data availability

Data and scripts are deposited in repositories, as listed in the manuscript.

The Illumina sequencing reads generated for this study have been submitted to the NCBI Sequence Read Archive (SRA) and can be retrieved through BioProject PRJNA1178623. GenBank Accessions for the mtDNA sequences are: PQ787233-PQ787287. All scripts utilized can be found in the following GitHub repository: https://github.com/Wielstra-Lab/Neurergus_phylogenomics

References

Alexander, D.H., Novembre, J., Lange, K., 2009. Fast model-based estimation of ancestry in unrelated individuals. Genome Res. 19 (9), 1655–1664. https://doi.org/10.1101/gr.094052.109.

Andermann, T., Torres Jiménez, M.F., Matos-Maraví, P., Batista, R., Blanco-Pastor, J.L., Gustafsson, A.L.S., Kistler, L., Liberal, I.M., Oxelman, B., Bacon, C.D., Antonelli, A., 2020. A guide to carrying out a phylogenomic target sequence capture project. Front. Genet. 10, 1407. https://doi.org/10.3389/fgene.2019.01407.

Arévalo, E., Davis, S.K., Sites, J.W., 1994. Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in Central Mexico. Syst. Biol. 43 (3), 387–418. https://doi.org/10.1093/sysbio/43.3.387.

Bouckaert, R., Vaughan, T.G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., Heled, J., Jones, G., Kühnert, D., De Maio, N., Matschiner, M., Mendes, F.K., Müller, N.F., Ogilvie, H.A., Du Plessis, L., Popinga, A., Rambaut, A., Rasmussen, D., Siveroni, I., Drummond, A.J., 2019. BEAST 2.5: an advanced software platform for bayesian evolutionary analysis. PLoS Comput. Biol. 15 (4), e1006650. https://doi.org/10.1371/journal.pcbi.1006650.

Cingolani, P., Patel, V.M., Coon, M., Nguyen, T., Land, S.J., Ruden, D.M., Lu, X., 2012. Using *Drosophila melanogaster* as a model for genotoxic chemical mutational studies with a new program. SnpSift. Frontiers in Genetics 3. https://doi.org/10.3389/fgene.2012.00035.

Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., McVean, G., Durbin, R., 2011. The variant call format and VCFtools. Bioinformatics 27 (15), 2156–2158. https://doi.org/10.1093/bioinformatics/btr330.

Danecek, P., Bonfield, J.K., Liddle, J., Marshall, J., Ohan, V., Pollard, M.O., Whitwham, A., Keane, T., McCarthy, S.A., Davies, R.M., Li, H., 2021. Twelve years of SAMtools and BCFtools. GigaScience 10 (2), giab008. https://doi.org/10.1093/gigascience/giab008.

De Visser, M.C., France, J., McCartney-Melstad, E., Bucciarelli, G.M., Theodoropoulos, A., Shaffer, H.B., Wielstra, B., 2024a. NewtCap: An efficient target capture approach to boost genomic studies in Salamandridae (True Salamanders and Newts). bioRxiv 2024.10.25.620290; https://doi.org/10.1101/2024.10.25.620290.

De Visser, M.C., France, J., Paulouskaya, O., Brown, T., Fahrbach, M., Van Der Ploeg, C., & Wielstra, B., 2024b. Conserved gene content and unique phylogenetic history characterize the 'bloopergene' underlying *Triturus*' balanced lethal system. bioRxiv 2024.10.25.620277; https://doi.org/10.1101/2024.10.25.620277.

Degnan, J.H., Rosenberg, N.A., 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. Trends Ecol. Evol. 24 (6), 332–340. https://doi.org/10.1016/j.tree.2009.01.009.

Dufresnes, C., Poyarkov, N., Jablonski, D., 2023. Acknowledging more biodiversity without more species. Proc. Natl. Acad. Sci. 120 (40), e2302424120. https://doi.org/10.1073/pnas.2302424120.

Ekblom, R., Galindo, J., 2011. Applications of next generation sequencing in molecular ecology of non-model organisms. Heredity 107 (1), 1–15. https://doi.org/10.1038/hdy.2010.152.

Farasat, H., Akmali, V., Sharifi, M., 2016. Population genetic structure of the endangered Kaiser's mountain newt, *Neurergus kaiseri* (Amphibia: Salamandridae). PLoS One 11 (2), e0149596. https://doi.org/10.1371/journal.pone.0149596.

Fleck, J., 2010. Beobachtungen an Irakischen Bergbachmolchen der Gattung Neurergus Cope, 1862 und Fragen zu ihrer taxonomischen Zuordnung nach der Wiederentdeckung der 'Forma typica'von 'Rhithrotriton derjugini Nesterov, 1916'. Elaphe 18 (4), 6.

France, J., De Visser, M.C., Arntzen, J.W., Babik, W., Cvijanović, M., Ivanović, A., Smith, J., Vučić, T., & Wielstra, B., 2024. Genomic evidence suggests the balanced lethal system in *Triturus* newts originated in an instantaneous speciation event. bioRxiv 2024.10.29.620207; https://doi.org/10.1101/2024.10.29.620207.

Frost, D.R., 2024. Amphibian Species of the World: an Online Reference. Version 6.2 (6-12-2024). Electronic Database accessible at. American Museum of Natural History, New York, USA. https://amphibiansoftheworld.amnh.org/index.php.

Gee, H., 2003. Ending incongruence. Nature 425 (6960), 782. https://doi.org/10.1038/425782a.

Goudarzi, F., Hemami, M.R., Malekian, M., Fakheran, S., Martínez-Freiría, F., 2021. Species versus within-species niches: a multi-modelling approach to assess range size of a spring-dwelling amphibian. Sci. Rep. 11 (1), 597. https://doi.org/10.1038/s41598-020-79783-0.

Goudarzi, F., Hemami, M.-R., Rancilhac, L., Malekian, M., Fakheran, S., Elmer, K.R., Steinfartz, S., 2019. Geographic separation and genetic differentiation of populations are not coupled with niche differentiation in threatened Kaiser's spotted newt (*Neurergus kaiseri*). Sci. Rep. 9 (1), 6239. https://doi.org/10.1038/s41598-019-41886-8.

Hendrix, R., Fleck, J., Schneider, W., Schneider, C., Geller, D., Avci, A., Olgun, K., Steinfartz, S., 2014. First comprehensive insights into nuclear and mitochondrial DNA based population structure of Near east mountain brook newts (Salamandridae: genus *Neurergus*) suggest the resurrection of *Neurergus derjugini*. Amphibia-Reptilia 35 (2), 173–187. https://doi.org/10.1163/15685381-00002939.

IUCN SSC Amphibian Specialist Group, 2016. *Neurergus kaiseri*: *IUCN SSC Amphibian Specialist Group: The IUCN Red List of Threatened Species 2016: E.T59450A49436271* [Dataset]. https://doi.org/10.2305/IUCN.UK.2016-3.RLTS.T59450A49436271.en.

IUCN SSC Amphibian Specialist Group, 2021. *Neurergus barani*: *IUCN SSC Amphibian Specialist Group: The IUCN Red List of Threatened Species 2023: E.T149686633A149686659* [Dataset]. https://doi.org/10.2305/IUCN.UK.2023-1.RLTS.T149686633A149686659.en.

IUCN SSC Amphibian Specialist Group. (2023a). *Neurergus crocatus*: *IUCN SSC Amphibian Specialist Group: The IUCN Red List of Threatened Species 2023: E.T14734A50980817* [Dataset]. https://doi.org/10.2305/IUCN.UK.2023-1.RLTS.T14734A50980817.en.

IUCN SSC Amphibian Specialist Group. (2023b). *Neurergus derjugini*: *IUCN SSC Amphibian Specialist Group: The IUCN Red List of Threatened Species 2023: E.T88373273A50759480* [Dataset]. https://doi.org/10.2305/IUCN.UK.2023-1.RLTS.T88373273A50759480.en.

IUCN SSC Amphibian Specialist Group. (2023c). *Neurergus strauchii*: *IUCN SSC Amphibian Specialist Group: The IUCN Red List of Threatened Species 2023: E.T149686691A149686856* [Dataset]. https://doi.org/10.2305/IUCN.UK.2023-1.RLTS.T149686691A149686856.en.

Kalaentzis, K., Arntzen, J.W., Avcı, A., Van Den Berg, V., Beukema, W., France, J., Olgun, K., Van Riemsdijk, I., Üzüm, N., De Visser, M.C., Wielstra, B., 2023. Hybrid zone analysis confirms cryptic species of banded newt and does not support competitive displacement since secondary contact. Ecol. Evol. 13 (9), e10442. https://doi.org/10.1002/ece3.10442.

Kalaentzis, K., Koster, S., Arntzen, J.W., Bogaerts, S., France, J., Franzen, M., Kazilas, C., Litvinchuk, S.N., Olgun, K., De Visser, M.C., Wielstra, B., 2025. Phylogenomics resolves the puzzling phylogeny of banded newts (genus *Ommatotriton*). https://doi.org/10.1016/j.ympev.2024.108237.

Kazilas, C., Dufresnes, C., France, J., Kalaentzis, K., Martínez-Solano, I., De Visser, M.C., Arntzen, J.W., Wielstra, B., 2024. Spatial genetic structure in European marbled newts revealed with target enrichment by sequence capture. Mol. Phylogenet. Evol. 194, 108043. https://doi.org/10.1016/j.ympev.2024.108043.

Khoshnamvand, H., Malekian, M., Keivani, Y., Goudarzi, F., 2019. DNA barcoding of the Luristan newt (*Neurergus kaiseri*) in south-western Iran. Journal of Wildlife and Biodiversity 3 (2). https://doi.org/10.22120/jwb.2019.34933.

Khoshnamvand, H., Malekian, M., Keivany, Y., 2018. Morphological distinction and sexual dimorphism in divergent clades of *Neurergus kaiseri* (Amphibia: Salamandridae). Basic and Applied Herpetology 32, 5–17. https://doi.org/10.11160/bah.102.

Khoshnamvand, H., Vaissi, S., Azimi, M., Ahmadzadeh, F., 2024. Phylogenetic climatic niche evolution and diversification of the *Neurergus* species (Salamandridae) in the Irano-Anatolian biodiversity hotspot. Ecol. Evol. 14 (8), e70105. https://doi.org/10.1002/ece3.70105.

Kopelman, N.M., Mayzel, J., Jakobsson, M., Rosenberg, N.A., Mayrose, I., 2015. Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. Mol. Ecol. Resour. 15 (5), 1179–1191. https://doi.org/10.1111/1755-0998.12387.

Kutschera, V.E., Bidon, T., Hailer, F., Rodi, J.L., Fain, S.R., Janke, A., 2014. Bears in a forest of gene trees: phylogenetic inference is complicated by incomplete lineage sorting and gene flow. Mol. Biol. Evol. 31 (8), 2004–2017. https://doi.org/10.1093/molbev/msu186.

Leaché, A.D., Banbury, B.L., Felsenstein, J., de Oca, A.N., Stamatakis, A., 2015. Short tree, long tree, right, tree wrong tree: new acquisition bias corrections for inferring SNP phylogenies. Syst. Biol. 64 (6), 1032–1047. https://doi.org/10.1093/sysbio/syv053.

Lewis, P.O., 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. Syst. Biol. 50 (6), 913–925. https://doi.org/10.1080/106351501753462876.
Lischer, H.E.L., Excoffier, L., 2012. PGDSpider: an automated data conversion tool for connecting population genetics and genomics programs. Bioinformatics 28 (2), 298–299. https://doi.org/10.1093/bioinformatics/btr642.
Lozano-Fernandez, J., 2022. A practical guide to design and assess a phylogenomic study. Genome Biol. Evol. 14 (9), evac129. https://doi.org/10.1093/gbe/evac129.
Malinsky, M., Matschiner, M., Svardal, H., 2021. Dsuite - fast *D*-statistics and related admixture evidence from VCF files. Mol. Ecol. Resour. 21 (2), 584–595. https://doi.org/10.1111/1755-0998.13265.
Mars, J., Koster, S., Babik, W., France, J., Kalaentzis, K., Kazilas, C., Martínez-Solano, Í., De Visser, M. C., & Wielstra, B., 2025. Phylogenomics yields new systematic and taxonomical insights for *Lissotriton* newts, a lineage with a strong legacy of introgressive hybridization. https://doi.org/10.1016/j.ympev.2024.108282.
Maurin, K.J.L., 2020. An empirical guide for producing a dated phylogeny with treePL in a maximum likelihood framework. https://doi.org/10.48550/ARXIV.2008.07054.
McCartney-Melstad, E., Mount, G.G., Shaffer, H.B., 2016. Exon capture optimization in amphibians with large genomes. Mol. Ecol. Resour. 16 (5), 1084–1094. https://doi.org/10.1111/1755-0998.12538.
McCormack, J.E., Hird, S.M., Zellmer, A.J., Carstens, B.C., Brumfield, R.T., 2013. Applications of next-generation sequencing to phylogeography and phylogenetics. Mol. Phylogenet. Evol. 66 (2), 526–538. https://doi.org/10.1016/j.ympev.2011.12.007.
Nader, I.A., 1969. The newt *Neurergus crocatus* COPE in Iraq. bull. bio. res. cent. Baghdad 4, 3–12.
Nikolskii, A.M., 1918. Fauna of Russia and Adjacent Countries. Amphibians. In Russian, English translation of 1962, Petrograd, 309 pp.
Olgun, K., Avci, A., Bozkurt, E., Üzüm, N., Olgun, H., Ilgaz, C., 2016. A new subspecies of Anatolia newt, *Neurergus strauchii* (Steindachner, 1887) (Urodela:Salamandridae), from Tunceli. Eastern Turkey. Russian Journal of Herpetology 23 (4), 271–277.
Ortiz, E.M., 2019. vcf2phylip v2.0: convert a VCF matrix into several matrix formats for phylogenetic analysis.
Özdemir, N., Üzüm, N., Avci, A., Olgun, K., 2009. Phylogeny of *Neurergus crocatus* and *Neurergus strauchii* in Turkey based on morphological and molecular data. Herpetologica 65 (3), 280–291. https://doi.org/10.1655/07-047R2.1.
Pabijan, M., Zieliński, P., Dudek, K., Stuglik, M., Babik, W., 2017. Isolation and gene flow in a speciation continuum in newts. Mol. Phylogenet. Evol. 116, 1–12. https://doi.org/10.1016/j.ympev.2017.08.003.
Pasmans, F., Bogaerts, S., Woeltjes, T., Carranza, S., 2006. Biogeography of *Neurergus strauchii barani* Öz, 1994 and *N. s. Strauchii* (Steindachner, 1887) (Amphibia: Salamandridae) assessed using morphological and molecular data. Amphibia-Reptilia 27 (2), 281–288. https://doi.org/10.1163/156853806777239878.
Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., De Bakker, P.I.W., Daly, M.J., Sham, P.C., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81 (3), 559–575. https://doi.org/10.1086/519795.
R Core Team (2024). R: A Language and Environment for Statistical Computing. RFoundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
Rambaut, A., Drummond, A.J., Xie, D., Baele, G., Suchard, M.A., 2018. Posterior summarization in bayesian phylogenetics using Tracer 1.7. Syst. Biol. 67 (5), 901–904. https://doi.org/10.1093/sysbio/syy032.
Rancilhac, L., Goudarzi, F., Gehara, M., Hemami, M.-R., Elmer, K.R., Vences, M., Steinfarz, S., 2019. Phylogeny and species delimitation of Near Eastern *Neurergus* newts (Salamandridae) based on genome-wide RADseq data analysis. Mol. Phylogenet. Evol. 133, 189–197. https://doi.org/10.1016/j.ympev.2019.01.003.
Rancilhac, L., Irisarri, I., Angelini, C., Arntzen, J.W., Babik, W., Bossuyt, F., Künzel, S., Lüddecke, T., Pasmans, F., Sanchez, E., Weisrock, D., Veith, M., Wielstra, B., Steinfartz, S., Hofreiter, M., Philippe, H., Vences, M., 2021. Phylotranscriptomic evidence for pervasive ancient hybridization among Old World salamanders. Mol. Phylogenet. Evol. 155, 106967. https://doi.org/10.1016/j.ympev.2020.106967.
RStudio Team (2024). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/.
Schmidt, K.P., 1955. Amphibians and reptiles from Iran. Videnskabelige Meddelelser Fra Dansk Naturhistorisk Forening i Kjøbenhavn 117, 193–207.
Schmidtler, J.J., Schmidtler, J.F., 1970. Morphologie, biologie und verwandtschaftsbeziehungen von *Neurergus strauchii* aus der Türkei. Senckenberg. Bio. 51 (1/2), 41–53.
Schmidtler, J.J., Schmidtler, J.F., 1975. Untersuchungen an westpersischen bergbachmolchen der gattung *Neurergus* (Caudata, Salamandridae). Salamandra 11 (2), 84–98.
Schneider, C., Schneider, W., 2011. Die bergbachmolche der gattung *Neurergus* im Irak. Herpetozoa 23 (3/4), 3–20.
Schneider, W., Schneider, C., 2013. Beobachtungen zur verbreitung der bergbachmolche *Neurergus derjugini* (Nesterov, 1916), *Neurergus microspilotus* (Nesterov, 1916) und *Neurergus kaiseri* schmidt, 1952 im Iran. Herpetozoa 26 (1), 27–38.
Sethuraman, A. (2014, November 13). Admixture maps in R for Dummies. The Molecular Ecologist. Retrieved September 15, 2022, from https://www.molecularecologist.com/2014/11/13/admixture-maps-in-r-for-dummies/.
Smith, S.A., O'Meara, B.C., 2012. treePL: divergence time estimation using penalized likelihood for large phylogenies. Bioinformatics 28 (20), 2689–2690. https://doi.org/10.1093/bioinformatics/bts492.
Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30 (9), 1312–1313. https://doi.org/10.1093/bioinformatics/btu033.
Steinfartz, S., Hwang, U.W., Tautz, D., Öz, M., Veith, M., 2002. Molecular phylogeny of the salamandrid genus *Neurergus*: evidence for an intrageneric switch of reproductive biology. Amphibia-Reptilia 23 (4), 419–431. https://doi.org/10.1163/15685380260462338.
Steinfartz, S., Weitere, M., Tautz, D., 2007. Tracing the first step to speciation: ecological and genetic differentiation of a salamander population in a small forest. Mol. Ecol. 16 (21), 4550–4561. https://doi.org/10.1111/j.1365-294X.2007.03490.x.
Stoltz, M., Baeumer, B., Bouckaert, R., Fox, C., Hiscott, G., Bryant, D., 2021. Bayesian inference of species trees using diffusion models. Syst. Biol. 70 (1), 145–161. https://doi.org/10.1093/sysbio/syaa051.
Stuglik, M.T., Babik, W., 2016. Genomic heterogeneity of historical gene flow between two species of newts inferred from transcriptome data. Ecol. Evol. 6 (13), 4513–4525. https://doi.org/10.1002/ece3.2152.
Vaissi, S., 2021. Historic range dynamics in Kaiser's mountain newt (*Neurergus kaiseri*): insights from phylogeographic analyses and species distribution modeling. Ecol. Evol. 11 (12), 7622–7633. https://doi.org/10.1002/ece3.7595.
Vaissi, S., Sharifi, M., 2021. The least-cost path analysis of landscape genetics identifies two dispersal routes for the threatened Kaiser's mountain newt (Caudata: Salamandridae). J. Zool. Syst. Evol. Res. 59 (7), 1491–1502. https://doi.org/10.1111/jzs.12510.
Vences, M., Miralles, A., Dufresnes, C., 2024. Next-generation species delimitation and taxonomy: implications for biogeography. J. Biogeogr. 51 (9), 1709–1722. https://doi.org/10.1111/jbi.14807.
Villesen, P., 2007. FaBox: an online toolbox for fasta sequences. Mol. Ecol. Notes 7 (6), 965–968. https://doi.org/10.1111/j.1471-8286.2007.01821.x.
Wang, Y., Nielsen, R., 2012. Estimating population divergence time and phylogeny from single-nucleotide polymorphisms data with outgroup ascertainment bias. Mol. Ecol. 21 (4), 974–986. https://doi.org/10.1111/j.1365-294X.2011.05413.x.
Weisrock, D.W., Papenfuss, T.J., Macey, J.R., Litvinchuk, S.N., Polymeni, R., Ugurtas, I. H., Zhao, E., Jowkar, H., Larson, A., 2006. A molecular assessment of phylogenetic relationships and lineage accumulation rates within the family Salamandridae (Amphibia, Caudata). Mol. Phylogenet. Evol. 41 (2), 368–383. https://doi.org/10.1016/j.ympev.2006.05.008.
Wielstra, B., Arntzen, J.W., 2011. Unraveling the rapid radiation of crested newts (*Triturus cristatus* superspecies) using complete mitogenomic sequences. BMC Evol. Biol. 11 (1), 162. https://doi.org/10.1186/1471-2148-11-162.
Wielstra, B., McCartney-Melstad, E., Arntzen, J.W., Butlin, R.K., Shaffer, H.B., 2019. Phylogenomics of the adaptive radiation of *Triturus* newts supports gradual ecological niche expansion towards an incrementally aquatic lifestyle. Mol. Phylogenet. Evol. 133, 120–127. https://doi.org/10.1016/j.ympev.2018.12.032.
Zhang, C., Rabiee, M., Sayyari, E., Mirarab, S., 2018. ASTRAL-III: polynomial time species tree reconstruction from partially resolved gene trees. BMC Bioinf. 19 (S6), 153. https://doi.org/10.1186/s12859-018-2129-y.
Zhang, P., Papenfuss, T.J., Wake, M.H., Qu, L., Wake, D.B., 2008. Phylogeny and biogeography of the family Salamandridae (Amphibia: Caudata) inferred from complete mitochondrial genomes. Mol. Phylogenet. Evol. 49 (2), 586–597. https://doi.org/10.1016/j.ympev.2008.08.020.