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CARNIOLAN LIZARD *ZOOTOCA CARNIOLICA* (REPTILIA: SQUAMATA: LACERTIDAE) IN CROATIA: IMPLICATION ON SPECIES CONSERVATION

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Accurate species distribution data are essential for effective conservation planning, which requires taxonomic identification. We conducted molecular and morphological analyses, which revealed that the Carniolan lizard *Zootoca carniolica* Mayer, Böhme, Tiedemann and Bischoff, 2000 has been largely overlooked in Croatia as records were previously attributed to the Viviparous lizard *Z. vivipara* (Lichtenstein, 1823) or the Pannonian viviparous lizard *Z. v. pannonica* (Lac & Kluch, 1968). *Z. vivipara* has been listed in the national fauna of Croatia, and *Z. v. pannonica* was listed as a species monitored and reported by Croatia under Article 17 of the European Habitat Directive. We analysed genetic (mitochondrial *COI* and *cytb* genes), morphological (differences in the ratio of mean ventral scale rows and number of femoral pores) and distributional data of the Croatian population. Our findings indicate that *Z. carniolica*, not *Z. vivipara*, is the species present in Croatia. However, given the limited sample size, we cannot exclude the possible occurrence of *Z. vivipara* in Croatia. Consequently, the occurrence of *Z. carniolica* entails conservation and management implications that differ from earlier assumptions. We emphasize the need for further studies involving a significantly larger number of samples to confirm these findings.

Keywords: Lacertidae, mt DNA, morphology, distribution, conservation

Grbac, I., Podnar, M., Lauš, B. & Schmidt, B.: Alpska gušterica *Zootoca carniolica* (Reptilia: Squamata: Lacertidae) u Hrvatskoj: implikacije za zaštitu vrste. Nat. Croat., Vol. 34, No. 2, 241-255, 2025, Zagreb.

Točni podaci o rasprostranjenosti vrsta ključni su za učinkovito planiranje zaštite, što podrazumijeva točnu taksonomsku identifikaciju. U ovom radu proveli smo molekularne i morfološke analize koje su pokazale da vrsta *Zootoca carniolica* Mayer, Böhme, Tiedemann & Bischoff, 2000 živi u Hrvatskoj. Raniji nalazi ove vrste pripisivani su živorodnoj gušterici *Z. vivipara* (Lichtenstein, 1823) ili panonskoj živorodnoj gušterici *Z. v. pannonica* (Lac & Kluch, 1968). *Z. vivipara* uvrštena je u nacionalnu faunu Hrvatske, dok je *Z. v. pannonica* navedena kao vrsta koju Hrvatska prati i o kojoj izvještava u skladu s člankom 17 Direktive o staništima. Analizirali smo genetske podatke (mitohondrijski geni COI i cytb), morfološke značajke (omjer prosječnog broja redova trbušnih ljuski i broja femoralnih pora) te podatke o rasprostranjenosti hrvatske populacije. Rezultati pokazuju da je u Hrvatskoj prisutna *Z. carniolica*, a ne *Z. vivipara*. Ipak, zbog ograničenog broja uzoraka, ne može se isključiti mogućnost da *Z. vivipara* također živi u Hrvatskoj. Potrebna su daljnja istraživanja na znatno većem broju uzoraka radi potvrde ovih nalaza. Nalazi o prisutnosti *Z. carniolica* upućuju na potrebu redefiniranja dosadašnjih smjernica zaštite i upravljanja.

Ključne riječi: Lacertidae, mtDNA, morfologija, rasprostranjenost, zaštita

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INTRODUCTION

Mistakes in species identification and consequent distribution may direct conservation efforts and resources wrongly and ultimately compromise the sustainable management and effective conservation of the target species (Vogel Ely *et al.*, 2017). Such examples are representatives of the genus *Zootoca* Wagler, 1830 which are small, cold-adapted lacertid lizards, widespread across Eurasia, reaching up to 60° N latitude, and classified as the northernmost lizard species (Sindaco & Jeremčenko, 2008). They are among the few squamates that possess an extremely rare bimodal reproductive mode, meaning that oviparity (egg-laying) and viviparity (live-bearing) occur in a single species over their geographical distribution (e.g. Whittington *et al.* 2022). In reptiles, viviparity often evolves as an adaptation to cold climates (Pyron & Burbrink, 2014; Shine, 2014).

Based on mitochondrial DNA markers, six genetic clades of *Z. vivipara*, have been described (Surget-Groba *et al.*, 2001, 2006; Horreo *et al.*, 2018), four of them viviparous and covering the majority of populations (clades C and F) and two oviparous (clades A and B). Oviparous populations inhabit the southernmost edge of the species distribution, clade A in the Alps (North Italy, Southern Austria, Slovenia and northwestern Croatia), and clade B in the Pyrenees (southern France and Northern Spain) (Meyer *et al.*, 2000; Surget-Groba *et al.*, 2006; Arribas, 2009; Horreo *et al.*, 2018). Oviparous populations were reclassified as subspecies: *Z. v. carniolica* (Mayer *et al.*, 2000) (clade A) and *Z. v. louislantzi* (Arribas, 2009) (clade B). Viviparous populations (clades C, D and F) belong to *Z. v. vivipara* (Cornetti *et al.*, 2015b) and clade E belongs to *Z. v. pannonica* (Lác & Kluch 1968; Surget Groba, 2006; Arribas, 2009). The seventh mitochondrial clade phylogenetically most closely related to the oviparous clade A and with an unreported reproductive mode, was discovered in the Carpathians (Romania, Făgăraş Mountains and Predeal) by Velekei *et al.* (2015).

Recently, *Z. carniolica* was accepted as a distinct species in the Species list of the European herpetofauna (Cornetti *et al.*, 15a, 15b; Speybroeck *et al.*, 2020). Other researchers suggested that genetic differences among *Z. v. vivipara* and *Z. v. carniolica* support their subspecies status (Mayer *et al.*, 2000; Horreo *et al.*, 2018, 2019; Whittington *et al.*, 2022 review). Although it was understood that morphologically these two lineages are similar (Guillaume *et al.*, 2006), recently a distinctive trait has been described (Rodriguez-Prieto *et al.*, 2017): the difference in the MVS / MFP ratio (mean ventral scale / mean femoral pores number).

In Croatia, only *Z. vivipara* (Jacquin, 1787) was recorded (Fig. 1) (Baškiera, 2013; Baškiera & Jelić, 2013; Jelić *et al.*, 2015; Lauš, 2016). Lowland populations from eastern Croatia were identified as *Z. v. pannonica* (Jelić & Bogdanović, 2011; Baškiera & Jelić, 2015), a European lowland steppe subspecies described from Slovakia (Lác & Kluch, 1968). Despite limited data, *Z. v. pannonica* was enrolled in the *Red book of Amphibians and Reptiles of Croatia* (Jelić *et al.*, 2015) as endangered species (EN) and was listed in the checklist of 32 reptile species that Croatia is monitoring and reporting under Article 17 of the Habitat Directive (Zadravec & Gambiroža, 2019)

The aim of this study was to produce the first comprehensive assessment of the genus *Zootoca* in Croatia using both morphological and genetic approaches to attribute populations to one species or the other. First, we aimed to prove which species are present in Croatia and second, whether the lowland populations belong to the subspecies *Z. v. pannonica*. We performed phylogenetic (mitochondrial *COI* and *cytb*

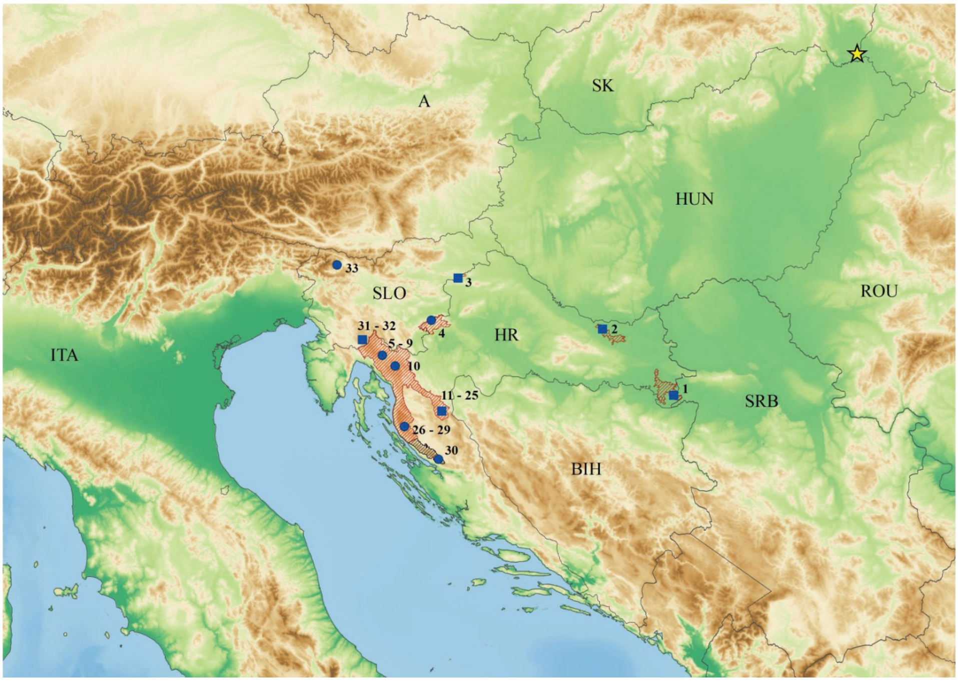

Fig. 1. The sampling locations of specimens analysed in the present study are marked by blue circles and those additionally analysed by genetic methodology by a blue square. The potential distribution is indicated by the red dashed surface. The type locality of *Z. v. pannonica* is market with a yellow star.
Abbreviations: A = Austria, AL = Albania, BG = Bulgaria, BIH = Bosnia and Herzegovina, GR = Greece, HR = Croatia, I = Italy, MNE = Montenegro, NMK = North Macedonia, RKS = Kosovo, SLO = Slovenia, SRB = Serbia, UA = Ukraine

genes) and morphological analyses in an attempt to determine the taxonomic status of several Croatian *Zootoca* populations. We analysed the presence, distribution and conservation implications of the *Zootoca* ssp. in Croatia.

MATERIALS AND METHODS

1. Sample collection

We analysed 33 samples (31 specimens and 2 tail tips) (Tab. 1) of *Zootoca ssp*. collected from 7 localities in Croatia, and 2 localities in Slovenia (Fig. 1). Morphological measurements were performed on 31 and genetic analyses on 5 specimens. Voucher specimens were preserved in 70% ethanol and deposited at the Croatian Natural History Museum (HPM), in the Collection of Amphibians and Reptiles (ZVG) and the Nina De Luca Collection of Amphibians and Reptiles (ZNDL). Lizard tissue, including tail and leg muscle, was obtained from specimens preserved in 70 – 75% ethanol (Tab. 1). All genetic analysis samples were also deposited in the Tissue Collection (TC) at the HPM.

Tab. 1. List of samples used in the study. Numbers in the 1st column refer to the numbers presented on the map in Fig. 1 Locality, Sex, Collection inventory numbers (ID; HPM – Croatian Natural History Museum, ZVG - Collection of the Amphibians and Reptiles, ZNL – Nina De Luca Collection of the amphibians and Reptiles, TC – Tissue Collection), **cytochrome c oxidase subunit I** (*COI*) and **cytochrome b** (*cytb*) GenBank sequence accession numbers, Latitude, Longitude and Altitude (ASL) are reported. * - original ASL data

No	Locality	Sex	Museum ID	Sample ID/ Accession Numbers (COI/cytb)/	Latitude	Longitude	ASL
1	HR_Jasenova, Spačva		TC3990	CROB1263 / CROHF004-23 / PP108655	44.982432	19.028361	80
2	HR_Breštanovci, Podravina		TC4288	ZOVI3 CROHF005-23 /PP108654	45.694657	17.994354	90
3	HR_Donji Macelj	m	ZVIG_4153	ZOVI2 CROHF003-23 /PP108653	46.239793	15.823812	280
4	HR_Pogane jame, Žumberak	f	ZVIG_3844		45.791491	15.431551	880
5	HR_Crni lug, Risnjak	m	ZNDL_1430		45.414053	14.704555	700*
6	HR_Crni lug, Risnjak	f	ZNDL_1431		45.414053	14.704555	700
7	HR_Leska, Crni lug, Risnjak	f	ZNDL_1460		45.415875	14.667944	700
8	HR_Crni lug, Risnjak	f	ZNDL_1432		45.414053	14.704555	700
9	HR_Vilje, Risnjak	f	ZNDL_1478		45.408890	14.6183	1180
10	HR_Begovo Razdolje, Gorski Kotar	f	ZVIG_70		45.304210	14.90021	1100
11	HR_Crna rijeka, Plitvička jezera	f	ZNDL_1470	CROB999 / CROHF001-23 / -	44.840427	15.599426	700
12	HR_Crna rijeka, Plitvička jezera	m	ZVIG_4060		44.840427	15.599426	700
13	HR_Crna rijeka, Plitvička jezera	m	ZVIG_4061		44.840427	15.599426	700
14	HR_Crna rijeka, Plitvička jezera	m	ZVIG_4062		44.840427	15.599426	700
15	HR_Crna rijeka, Plitvička jezera	m	ZNDL_1461		44.840427	15.599426	700
16	HR_Crna rijeka, Plitvička jezera	m	ZNDL_1462		44.840427	15.599426	700
17	HR_Crna rijeka, Plitvička jezera	m	ZNDL_1463		44.840427	15.599426	700
18	HR_Crna rijeka, Plitvička jezera	m	ZNDL_1464		44.840427	15.599426	700
19	HR_Crna rijeka, Plitvička jezera	f	ZNDL_1465		44.840427	15.599426	700
20	HR_Crna rijeka, Plitvička jezera	f	ZNDL_1466		44.840427	15.599426	700
21	HR_Crna rijeka, Plitvička jezera	m	ZNDL_1469		44.840427	15.599426	700
22	HR_Crna rijeka, Plitvički Ljeskovac, Plitvice	m	ZNDL_1473		44.840427	15.599426	700*
23	HR_Crna rijeka, Plitvički Ljeskovac, Plitvice	m	ZNDL_1474		44.840427	15.599426	700
24	HR_Crna rijeka, Plitvički Ljeskovac, Plitvice	f	ZNDL_1475		44.840427	15.599426	790*
25	HR_Plitvice, Plitvička jezera	f	ZVIG_2268		44.899167	15.593333	580
26	HR_Crni Padež, Štirovača, Velebit	f	ZVIG_69		44.671728	15.05203	1150
27	HR_Crni Padež, Štirovača, Velebit	f	ZVIG_519		44.671728	15.05203	1150
28	HR_Mrkvište, Štirovača, Velebit	m	ZVIG_68		44.709444	15.043056	1280
29	HR_Štirovača, Velebit	f	ZVIG_67		44.709444	15.043056	1280
30	HR_Sveto Brdo, Velebit	f	ZVIG_66		44.333871	15.561382	1500
31	SLO_Sviščaki, Snežnik, Ilirska Bistrica	f	ZNDL_1476	CROB1001 / CROHF002-23 / -	45.574220	14.402097	1200*
32	SLO_Mrzli Studenec below Triglav	f	ZVIG_1993		46.349722	13.990833	1280
33	SLO_Sviščaki, Snežnik, Ilirska Bistrica	f	ZNDL_1477		45.574220	14.402097	1250*

2. Morphological analysis

We analysed 31 adult specimens (13 males and 18 females) from the viewpoint of morphology. Specimens were classified as adults if their SVL was equal to or greater than 45 mm for males and 48 for females (Roitberg *et al.*, 2020). The counts were performed by a single operator to eliminate any potential operator-dependent bias. We measured snout-vent length (SVL), number of femoral pores (FP) and number of ventral scales (VS) counted longitudinally along intermediate left and right rows. SVL was measured using a digital calliper (Mitutoyo) to the closest 0.1 mm. FP were counted on the right and left limb and the average between them was calculated (MFP). Ventral scales were counted along the median longitudinal line, starting from the cloaca, from the first row of scales showing four sub-rectangular scales up to the collar (Guillaume *et al.*, 2006; Rodriguez-Prieto *et al.*, 2017) (Fig. 2). Ventral scales were counted twice, once on each side of the median longitudinal line and the average was recorded (MVS, Fig. 2). The raw data can be found in the Appendix 1 (Supplementary material 1).

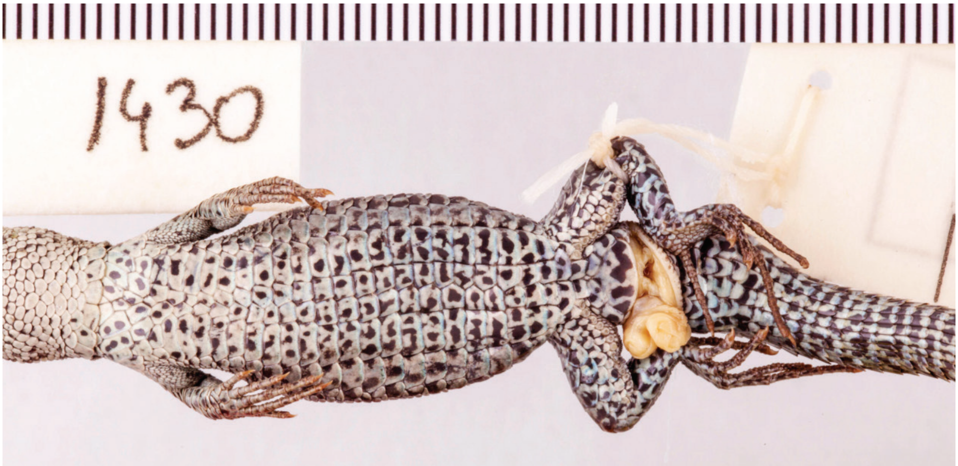

Fig. 2. Study specimen of *Zootoca carniolica* from the Nina De Luca Collection of Amphibians and Reptiles (ZNDL), with the start of the ventral scale count marked (red dash).

3. DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from 2 mm long, ethanol-preserved tail tips of five specimens (Tab. 1) using the GeneElute Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich). The standard DNA barcode region, a 658-base pair fragment of the 5′ end of the mitochondrial cytochrome c oxidase subunit 1 (*COI*) gene, was amplified for all five samples using dgLCO-1490 and dgHCO-2198 amplification primers (Meyer, 2003). To enable the assignment of our samples to previously identified major mitochondrial lineages (e. g. Surget-Groba *et al.*, 2006; Cornetti *et al.*, 2014; Velekei *et al.*, 2015), the complete mitochondrial cytochrome *b* gene (*cytb*) was amplified for three samples (Tab. 1) using LGLuLK and NTheH primers (Pavlicev & Mayer, 2009). For both markers, the 20 µl PCR mix incorporated 0.625 U of DreamTaq DNA Polymerase (Thermo Fisher Scientific), 1× DreamTaq Green Buffer, 200 µM of each dNTP, 0.4 μM of each primer, and 5-10 ng of DNA template. The PCR cycling parameters were as follows: an initial denaturation at 95°C for 2 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 60 seconds for *COI* or 90 seconds for *cytb* amplification. The final extension was performed at 72°C for 7 minutes. Sequencing was carried out by Macrogen Europe (The Netherlands) using the amplification primers. BOLD process IDs and GenBank accession numbers are given in Tab. 1.

4. Datasets and sequence alignments

The *Z. vivipara* and *Z. carniolica cytb* sequences were downloaded from GenBank, and *COI* sequences were retrieved from BOLD (www.boldsystems.org; Ratnasingham & Hebert, 2007) in December 2023. The cytb data were organised into three datasets. Along with the three sequences obtained in the present study, *Cytb dataset1* included all publicly available *cytb* sequences of varying length (Appendix 2, Supplementary material S2). The reduced, *Cytb dataset2*, contained the newly obtained sequences, as well as representatives from each of the seven currently recognised main mitochondrial clades (six clades of *Z. vivipara* and *Z. carniolica* clade), including the sequence of the specimen from the type locality (*terra typica, tt*) of *Z. v. pannonica* (CB-4, Recknagel *et al.*, 2018). *Cytb dataset3* comprised available *Z. carniolica* sequences longer than 382 bp. Finally, the COI data set included all publicly accessible DNA barcode sequences of *Zootoca*, and five newly obtained sequences from this study. The sequence of *Iberolacerta horvathi* (Méhely, 1904) was used as an outgroup (Recknagel *et al.*, 2018). Sequence alignments were performed online using Mafft version 7 (Katoh & Standley, 2013) with default settings.

5. Phylogenetic analyses

Phylogenetic analyses were performed using Neighbor-Joining (NJ) and Bayesian Inference (BI) approaches. A preliminary phylogenetic analysis was conducted on *Cytb dataset1* using the NJ method as implemented in the MEGA version 11.0.13 (Kumar *et al.*, 2018) with uncorrected (p) distances and pairwise deletion options. Branch support was assessed using 2000 bootstrap replicates. The same analysis, under the same conditions, was then performed on *Cytb dataset2* and *COI* dataset, with a single difference: for the latter, the Kimura 2-parameter (K2P; Kimura, 1980) distance model was used.

For the BI based on *Cytb dataset2* and *COI dataset*, a partitioning scheme by codon positions was applied, and the optimal model of evolution for each codon position was selected using jModelTest 0.1.1 (Posada, 2008) under the Bayesian information criterion (BIC). Analyses were performed using MrBayes (v.3.2.6, Huelsenbeck & Ronquist, 2001; Ronquist *et al.*, 2012) under the selected evolutionary models. Two independent parallel runs of four Monte Carlo Markov Chains (MCMCs) were conducted for 10,000,000 generations, sampling every 1000th generation. Tracer v1.7.1 (Rambaut *et al.*, 2018) was used to assess convergence and effective sample size (ESS) values. After the first 25% of sampled trees were discarded as burn-in, the 50% majority-rule consensus tree with nodal values representing Bayesian posterior probabilities (PP) was obtained from the remaining trees.

Finally, the statistical parsimony network of *Z. carniolica* haplotypes (*Cytb dataset3*) was reconstructed using TCS algorithm (Clement *et al.*, 2022) as implemented in PopART v. 1.7 software (Leigh & Bryant, 2015) with default settings.

RESULTS

Morphological analyses

Sample statistics of the morphological analysis are shown in Tab. 1. Females had a longer body (SVL) and a greater number of ventral scales (MVS), on average, than males. The average number of femoral pores (MFP) was slightly higher in males than in females (Tab. 2). All males had MVS/MFP ratios lower than 2.2, a feature attributed to *Z. carniolica*, while 16 females had MVS/MFP ratios lower than 2.45 which is characteristic of female *Z. carniolica*. Two females had ratios higher than 2.48, a feature proposed for *Z. vivipara* (Rodriguez-Prieto *et al.*, 2017).

Tab. 2. Sample statistics for *Z. carniolica*; The mean and standard error of snout-vent length body (SVL), number of ventral scales (MVS), femoral pores (MFP) and MVS/MFP ratio of males and females are shown.

Sex	No	SVL ±SE (mm)	MVS ±SE	MFP ±SE	MVS/MFP ±SE
Male	13	55.0 ±1.0	23.4 ±0.2	12.7 ±0.3	1.9 ±0.04
Female	18	56.9 ±1.4	25.5 ±0.2	12.2 ±0.3	2.1 ±0.05
All	31	56.1 ±0.9	26.6 ±0.3	12.4 ±0.2	2.0 ±0.04

Phylogenetic analyses

The phylogenetic analyses based on both the *COI* and the *cytb* gene, place all samples from this study within the well-supported *Z. carniolica* clade (Figs. 3 and 4). Additionally, samples from Donji Macelj and Spačva (localities 3 and 1, Fig. 1) share the previously published *cytb* haplotype OS1 (Surget-Groba *et al.*, 2002), while the haplotype from the Podravina sample is newly obtained and differs from the previous one by a single mutational step (Fig. 5).

DISCUSSION

We confirmed the presence of *Z. carniolica* in Croatia, currently representing the southernmost limit of its known distribution. Molecular analyses in this study identified all specimens as *Z. carniolica* (clade A). Morphological data were largely consistent, with 29 out of 31 specimens attributed to *Z. carniolica*. Previous reports had misidentified Croatian lowland populations as *Z. v. pannonica*; however, our molecular analyses clearly demonstrate that these populations belong to *Z. carniolica*. Although we could not confirm the presence of *Z. vivipara*, our sample is limited and so does not allow us to rule out its presence in Croatia.

Three Croatian populations exhibited two haplotypes; samples from Donji Macelj and Spačva, shared the OS1 haplotype also found in the northeast Slovenian and Italian populations (Surget-Groba *et al.*, 2002), while a newly identified haplotype from Podravina (ZOVI3, Fig. 5) differed by a single mutational step. The presence of *carniolica* mitochondrial haplotypes in the Podravina and Donji Macelj populations suggests a past or existing connection between these now-fragmented populations. However, the low genetic diversity observed may be a result of the small sample size, precluding definitive conclusions. Further genetic research with larger sample sizes is necessary.

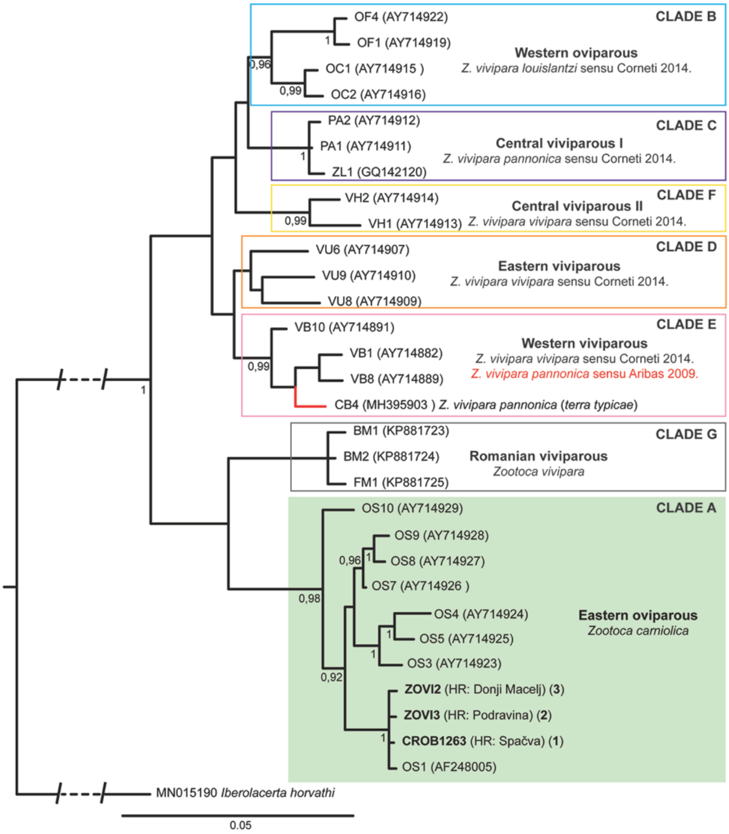

Fig. 3. Bayesian phylogram based on *cytb* sequences. Numbers at nodes represent Bayesian posterior probabilities (>0.9).

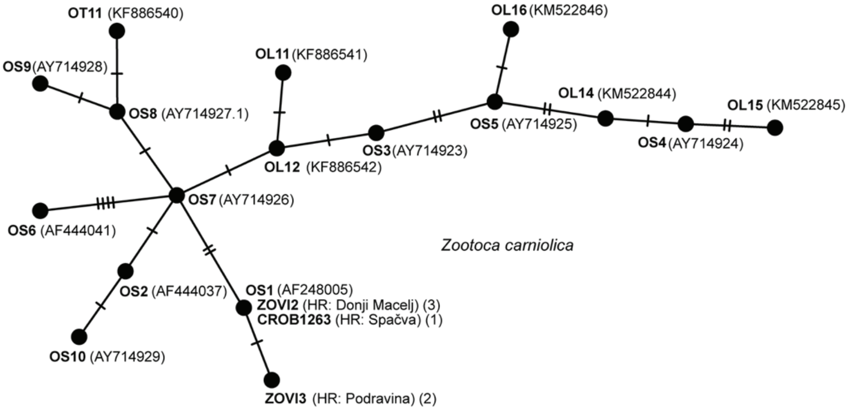

Fig. 4. Phylogram based on Neighbour-Joining analysis of DNA barcode sequences (mitochondrial *COI* gene). Numbers at nodes represent boot-strap values (>70).

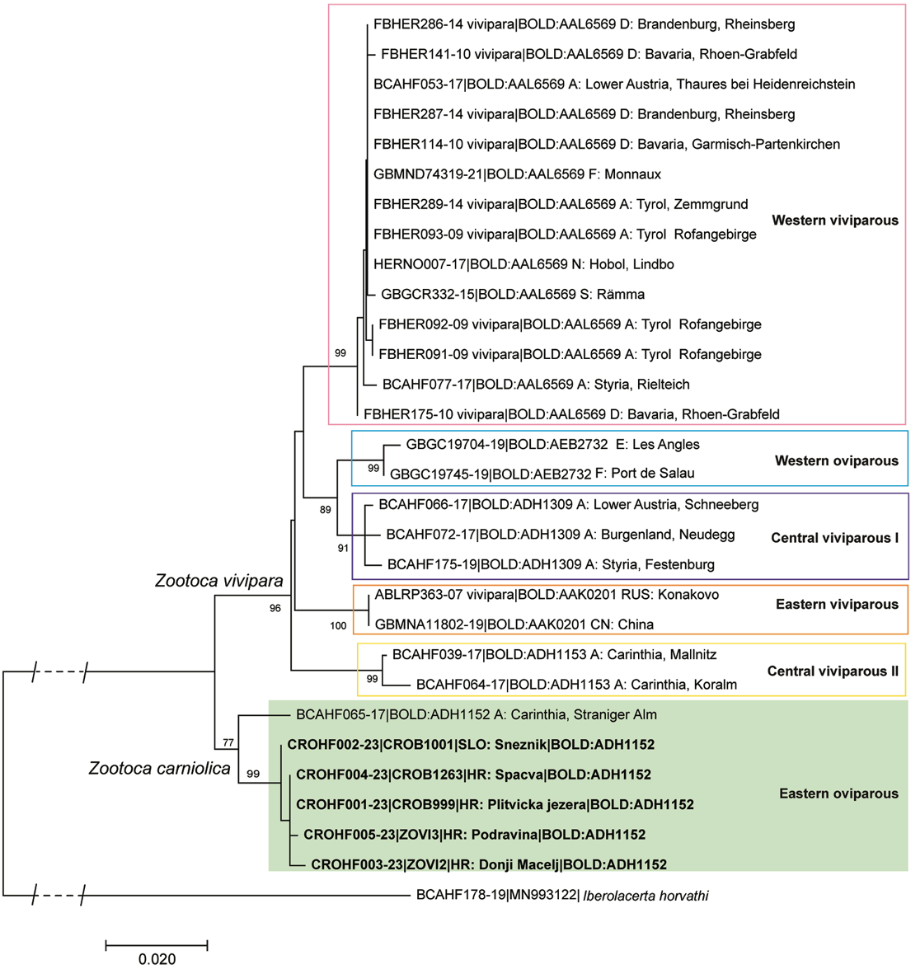

Fig. 5. Statistical parsimony network of *Z. carniolica* haplotypes based on the partial (382 bp) *cytb* sequences.

A key difference between *Z. carniolica* and *Z. vivipara* is their reproductive mode.

Heulin *et al*. (2000) identified the ATA^{-}200 allele of aspartate transaminase (AST) in the Plitvice Lakes population, indicating oviparity. Baškiera (2013) confirmed oviparity in the Spačva population by observing egg-laying in six gravid females. Given the significant ecological implications of reproductive mode, this information is critical for developing effective conservation strategies. It was considered that oviparous and viviparous lineages are morphologically very similar making them difficult to distinguish solely on external features. Guillaume *et al.* (2006) found that although these lineages cannot be unequivocally distinguished based on overall morphology, they do differ in several pholidosis characteristics. Their sample of *Z. v. carniolica* (Slovenia & Italy) differed from other samples of the species in having fewer ventral scales rows, more femoral pores and lower values of the D10 index. Rodríguez-Prieto *et al.* (2017)

introduced the MVS/MFP ratio (mean ventral scale rows/mean femoral pores) as a diagnostic feature: *Z. carniolica* males <2.20 and females <2.45; *Z. vivipara* males >2.27 and females >2.48. In our sample, all males and 16 females matched *Z. carniolica* ratios. Two females had a ratio that corresponded to *Z. vivipara*. The first female (no. 19, ZNDL 1465, Tab. 1, Fig. 1.) was sampled on Crna Rijeka, Plitvice HR. All other specimens collected at that location match *Z. carniolica* by MVS/MFP ratio, as does the one specimen that was genetically analysed (no. 5, ZVIG 1430, Tab. 1, Fig. 1). The second female (no. 32, ZVIG 1993, Tab. 1, Fig. 1) originates from "below Triglav", that is, from a locality in the northwest of Slovenia. Specimens from nearby areas were classified as *Z. vivipara* (Central viviparous 2, F clade, ELT03935 from Mojstrana, Recknagel *et al.*, 2018.) or *Z. carniolica* (Eastern viviparous, clade A, OSL5A from Zelenci, Horreo *et al.*, 2018). Genetic analysis was not possible for these two specimens due to DNA degradation.

The Pannonian viviparous lizard *Z. v. pannonica* was originally described from the eastern Slovakian lowlands (Boťany, Kapušanský Forest) based on morphology and lowland occurrence (Lác & Kluch, 1968). The name "pannonica" was subsequently applied uncritically to various Pannonian populations, though genetic and karyological studies show significant heterogeneity (Mayer, 2015). Phylogenetic studies (Surget-Groba, 2001, 2006; Arribas, 2009) revealed that the type specimen belongs to the Western Viviparous clade (clade E), not closely related to other so-labelled populations. Our study corroborates this classification using the type locality sequence (CB4, Recknagel *et al.*, 2018). Despite Arribas (2009) having corrected the clade nomenclature, Cornetti (2014) mistakenly associated clade C with *Z. v. pannonica*. Earlier Croatian reports misidentified lowland populations as *Z. v. pannonica* (Jelić & Bogdanović, 2011; Baškiera & Jelić, 2015). Baškiera (2013) questioned this classification based on reproductive evidence. Phylogenetic analyses based on mitochondrial DNA markers suggested that the Croatian populations investigated here belong to *Z. carniolica*.

The Carniolan lizard is endemic to Europe, occurring in northeastern Italy, southern Austria, Slovenia, northwestern Croatia (Mayer *et al.*, 2000; Kupriyanova *et al.*, 2006, Lindtke *et al.*, 2010; Cornetti *et al.*, 2014, 2015a; Jelić *et al.*, 2016) and Switzerland (Dufresnes *et al.*, 2021). The range of this recently recognised species is not well understood at present, and identification of its distribution in Croatia still requires work. The species lives in montane areas, in the northwest parts of Croatia: Gorski Kotar, Velebit, Plitvička jezera, Žumberak and Macelj. Lowland populations are so far known from eastern Croatia: Spačva and surroundings and here we reporta newly found lowland population in Podravina (NE Croatia). The reports from Mt Papuk (Barišić & Bogdanović, 2011; Radovanović, 1951) have not been documented, or confirmed by recent research (Burić *et al.*, 2023). Specimen ZVG_66 (no. 30 on Fig. 1) from Sveto Brdo on southern Velebit is somehow out of the species distribution and has not been confirmed in this area. Unlike Papuk, here we have an actual museum voucher specimen, so the presence of the species was marked on the distribution map. Croatian populations of *Z. carniolica* inhabit cool, moist, shaded microclimates, ranging from below 100 m a.s.l. in lowlands to 1250 m a.s.l. in montane zones (Fig. 6).

Reptile species face increased threats from climate change (Sinervo *et al.* 2010) particularly cold-adapted, hydrophilic species like *Z. carniolica* and *Z. vivipara*. Although lowland populations could theoretically migrate to higher elevations, suitable habitats may already be occupied, increasing competition. Garcia-Porta *et al.* (2019) identified

Fig. 6. Male and female *Zootoca carniolica* and habitat in Spačva (eastern Croatia). Photo: B. Lauš.

mountain specialists as especially vulnerable among lacertids, in addition to tropical thermoconformers that already live near their thermal maximum. Among lacertids, *Z. vivipara* has the highest evaporative water loss, and typically inhabits moist habitats that provide constant access to water (Dupoé *et al.*, 2017). Further, *Z. vivipara* is an accurate and effective thermoregulator (e.g. Bauwens *et al.*, 1996; Gvoždík *et al.*, 2002). As water availability may affect behavioural thermoregulation (Lorenzon *et al.*, 1999), in too dry conditions lizards may be forced to thermoregulate at suboptimal conditions or become inactive (Sinervo *et al.*, 2010).

From the current perspective *Z. carniolica* is a threatened species. Lowland populations from Italy are largely extinct (Giovone *et al.*, 2016; Cornetti, 2018), surviving only in a few central Po plain refuges. Their reliance on bogs and marshes makes them vulnerable to habitat destruction and climate change. Deforestation further reduces shaded microclimates. A study of alpine populations in northern Italy showed strong ecological differentiation between *Z. vivipara* and *Z. carniolica* due to their different reproduction mode (Cornetti, 2015b). Oviparous *Z. carniolica* preferred warmer, wetter, and more productive habitats (Cornetti, 2015b; Giovone *et al.*, 2016). It primarily occupied montane and humid pastures at medium altitudes, which are now threatened by increasing woodland encroachment due to the abandonment of traditional grazing practices (Giovine *et al.*, 2016). Croatian lowland populations are fragmented and highly pressured, while data on montane populations remain scarce. Regardless of taxonomic status, *Z. carniolica* is an evolutionarily significant unit due to its distinct reproductive mode, karyotype, and mtDNA. Focused distributional and ecological research is needed for effective conservation.

We propose the Croatian name "alpska gušterica" (Alpine lizard) for *Z. carniolica*, reflecting its Alpine origin. The species' Pleistocene glacial refugia was in the South of the Alps (Horreo & Fitze, 2022), followed by an expansion to the east. In other words, in Croatia, *Z. carniolica* came from the Alps and most of the species' distribution is in the Alps.

In conclusion, this study confirms the presence of *Zootoca carniolica* in Croatia, extending its known range to the south. Although *Z. vivipara* was not detected, its presence cannot be ruled out. *Z. carniolica* inhabits cool, moist habitats and is vulnerable to climate change and habitat loss, especially in lowland areas. Due to its distinct evolutionary traits and fragmented distribution, the species should be considered a conservation priority. We recommend increased ecological and genetic research to support its preservation.

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SUPPLEMENT MATERIAL

S1. Appendix 1. List of samples with morphological measurements. Also, the counts of the ventral scales on the left (LVS) and right (RVS) side, as well as the mean value (MVS) are reported. Moreover, the counts of the femoral pores on the left (LFP) and right (RFP) limb, as well as the mean value (MFP) are reported. MVS/MFP indicates the ratio MVS over MFP.

S2. Appendix 2. Phylogram based on Neighbor-Joining analysis of all publicly available cytb sequences of varying length. Sequences from this study are highlighted. Numbers at nodes represent boot-strap values.