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Species Delimitation in Morphologically Conserved Lizards

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ABSTRACT

Morphological conservatism in lacertid lizards generates extensive cryptic species diversity that is systematically underestimated by traditional taxonomy, with conservation consequences for species classified as widespread and common that may in fact comprise multiple range-restricted endemics. This study applied an integrative taxonomic framework combining mitochondrial phylogenetics (cytochrome b, ND4; 2,840 bp), genome-wide SNP genotyping (RADseq; 8,420 loci from 284 individuals), multivariate morphometrics (38 scalation and biometric characters), and ecological niche modelling (MaxEnt) to delimit species within the Lacerta viridis complex across 42 sampling localities in Central and Southern Europe. Mitochondrial phylogenetic analysis recovered eight well-supported lineages (bootstrap support > 84% in all cases; mean uncorrected p-distance between lineages 4.8 +/- 0.8%), exceeding the 2% barcoding threshold for species-level divergence in all pairwise comparisons. STRUCTURE analysis of RADseq SNPs identified K = 7 genomic clusters with mean pairwise FST of 0.184 +/- 0.042. Discriminant analysis of morphometric characters correctly reclassified 82.4% of individuals to their molecular lineage group (cross-validated). Ecological niche models showed significant niche divergence between six of eight lineage pairs (Schoener's D < 0.40). Applying the general lineage concept of species delimitation, we recognise six species as diagnosable units supported by congruent molecular, morphological, and ecological evidence, including the formal description of two lineages as new species. These results increase the known species richness of European lacertids by two and identify three single-locality endemic lineages requiring immediate conservation status assessment.

Keywords: species delimitation; cryptic species; lacertid lizards; Lacerta viridis complex; integrative taxonomy; RADseq; mitochondrial phylogenetics; morphometrics; ecological niche modelling; general lineage concept; European herpetofauna; conservation

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1. Introduction

1.1 Cryptic Species and the Taxonomy Gap

Cryptic species -- morphologically near-identical but genetically distinct lineages that have diverged without detectable morphological differentiation -- represent one of the most pervasive sources of underestimation of biodiversity, particularly in taxa with conservative bauplan and strong stabilising selection on external morphology (Bickford et al. 2007). Squamate reptiles, and lacertid lizards in particular, are disproportionately represented among vertebrate taxa found to conceal cryptic diversity: the genus Lacerta alone has seen its recognised species count more than double since molecular methods were first systematically applied to the group in the 1990s (Arnold 1989; Fu 2000). The conservation significance is substantial -- species assessed as widespread under traditional taxonomy may comprise multiple narrow endemics, each with restricted ranges and small populations that independently merit threatened status under IUCN criteria (Fitzpatrick et al. 2011).

1.2 Integrative Taxonomy

The integrative taxonomy framework, formalised by Dayrat (2005) and Padial et al. (2010), addresses the limitations of single-character species delimitation by combining evidence from multiple independent data sources -- molecular phylogenetics, population genomics, morphometrics, ecology, and geography -- to assess whether putative lineages meet the criteria for species recognition under a chosen species concept. The general lineage concept (de Queiroz 2007) provides the theoretical basis: independently evolving metapopulation lineages are species, with operational criteria including reciprocal monophyly, diagnostic morphological characters, ecological differentiation, and absence of ongoing gene flow serving as lines of evidence rather than necessary conditions. Congruence across multiple independent data types provides the strongest justification for species recognition.

1.3 The Lacerta viridis Complex

The western green lizard Lacerta viridis (Laurenti 1768) was long treated as a single widespread Palearctic species distributed from France to Turkey, but molecular studies by Rykena (1991) and Schmidtler (1997) first suggested deep genetic subdivision within the complex. Subsequent work by Joger et al. (1997) and Malhotra and Thorpe (2004) identified multiple divergent mtDNA lineages but lacked the genomic resolution and geographic sampling density to provide definitive species-level conclusions. The complex remains a priority target for integrative taxonomic revision given its conservation relevance (several populations protected under the EU Habitats Directive Annex IV), the availability of extensive historical museum material, and the practical challenges of surveying morphologically similar taxa in the field.

1.4 Study Objectives

This study aimed to: (i) characterise mitochondrial genetic diversity and phylogenetic structure across 42 sampling localities spanning the Lacerta viridis complex range; (ii) quantify genome-wide population structure using RADseq SNP genotyping; (iii) test for morphological diagnosability of molecular lineages using multivariate morphometrics; (iv) assess ecological niche divergence between lineage pairs; and (v) apply integrative species delimitation criteria to formally revise the species-level taxonomy of the complex.

2. Literature Review

2.1 Molecular Species Delimitation Methods

Pons et al. (2006) introduced the GMYC (General Mixed Yule-Coalescent) model as the first likelihood-based molecular clock method for species delimitation from ultrametric gene trees, identifying the transition from inter-specific branching (Yule process) to intra-specific coalescence as the operational species boundary. The bPTP (Bayesian Poisson Tree Process) model of Zhang et al. (2013) improved on GMYC by operating on non-ultrametric trees and providing Bayesian support values for each delimited entity. Population genomic approaches using RADseq-derived SNPs and STRUCTURE-based clustering (Evanno et al. 2005) or Treemix modelling (Pickrell and Pritchard 2012) provide complementary evidence of reproductive isolation that is independent of the mitochondrial genealogy, overcoming the single-locus limitations of mtDNA-based delimitation (Shaffer and Thomson 2007).

2.2 Morphometrics in Lacertid Taxonomy

Traditional lacertid taxonomy relies heavily on scalation characters -- the number of dorsal scales, femoral pores, subdigital lamellae, and supralabial, infralabial, and supratemporal scales -- which show diagnostic variation between some species pairs but high overlap in morphologically

conserved complexes (Arnold 1989). Geometric morphometrics applied to head shape has shown greater discriminatory power in some groups (Kaliontzopoulou et al. 2010), while linear body proportion ratios (head length/body length, hindlimb/body length) provide additional characters that correlate with microhabitat use and can differ between syntopic species. Multivariate discriminant analysis combining scalation counts with biometric ratios has been the most successful approach for morphological species diagnosis in European lacertids (Sillero et al. 2014).

2.3 Ecological Niche Modelling in Species Delimitation

Ecological niche modelling (ENM) using MaxEnt (Phillips et al. 2006) contributes to integrative taxonomy by testing whether putative species occupy distinguishable regions of environmental space -- a prediction of the ecological differentiation model of speciation (Nosil 2012). Niche overlap statistics including Schoener's D and Warren's I (Warren et al. 2008) quantify the degree of environmental similarity between pairs of lineage distributions, with values < 0.40 generally interpreted as indicating significant niche divergence. In Mediterranean lizard complexes, ENM-based niche divergence has been found to parallel genetic divergence and to predict areas of secondary contact where range expansions after Pleistocene glacial refugia brought previously allopatric lineages into contact (Leache et al. 2009).

2.4 Conservation Implications of Cryptic Diversity

Recognition of cryptic species within formerly widespread taxa typically elevates the conservation concern for the component lineages: a species with a 200,000 km2 distribution is not of conservation concern, while two species each occupying 100,000 km2 of that range may each qualify as Near Threatened under IUCN criterion B (Bickford et al. 2007; Fitzpatrick et al. 2011). For European lacertids specifically, cryptic species recognised within formerly widespread taxa frequently qualify for Habitats Directive Annex IV listing under the EU's legal framework for species protection, triggering requirements for population monitoring, habitat management, and impact assessment across member state territories (Sillero et al. 2014).

Table 1. Sampling Localities, Sample Sizes, and Lineage Assignments

Country	Localities (n)	Individuals (n)	Lineages Detected	Novel Lineage	EU Habitats Directive
Germany	6	38	I, II	No	Annex IV listed
Austria	4	28	I, II, III	No	Annex IV listed
Czech Republic	3	22	II	No	Annex IV listed
Hungary	4	26	II, IV	No	Not listed
Romania	5	34	IV, V	No	Annex IV listed
Bulgaria	5	38	V, VI	No	Not listed
Greece	6	42	VI, VII	VII = new sp.	Not listed
Turkey (Thrace)	4	28	VII, VIII	VIII = new sp.	Not listed
Italy (NE)	5	28	I, III	No	Annex IV listed
Total	42	284	8 lineages	2new species	

Lineage assignments from STRUCTURE K=7 + mtDNA phylogeny. Lineages I-VI correspond to currently recognised taxa; lineages VII and VIII are newly delimited species. EU Habitats Directive status applies to lineages previously assigned to Lacerta viridis sensu lato.

3. Materials and Methods

3.1 Tissue Sampling and DNA Extraction

Tissue samples (tail tips, 0.5-1.0 cm) were collected from 284 adult lizards across 42 localities in nine countries between 2019 and 2023, stored in 96% ethanol at -20 degC. Museum specimens (n = 68) from 14 additional historically important localities were accessed from the Museo Civico di Storia Naturale di Milano, the Naturkundemuseum Berlin, and the Hungarian Natural History Museum Budapest. Total genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen) following the standard protocol with extended lysis (56 degC, 3 hours) for museum specimens. DNA quality and concentration were assessed by NanoDrop spectrophotometry and Qubit fluorometric quantification.

3.2 Mitochondrial Sequencing and Phylogenetic Analysis

Cytochrome b (1,140 bp) and ND4 (1,700 bp) were amplified using published primers (Joger et al.

1997; Mayer and Beyerlein 2002) and Sanger-sequenced at Macrogen Europe (Amsterdam). Sequences were assembled and aligned in Geneious v2023 (Kearse et al. 2012). Maximum likelihood phylogenetic analysis was conducted in IQ-TREE v2.2 (Minh et al. 2020) with ModelFinder best-fit substitution model (GTR+G+I selected for both partitions). Bayesian inference was performed in MrBayes v3.2 (Ronquist et al. 2012) with four chains, 10 million generations, and 25% burn-in. Uncorrected p-distances between lineages were computed in MEGA v11 (Tamura et al. 2021). bPTP species delimitation was applied to the ML tree in the bPTP web server (Zhang et al. 2013).

3.3 RADseq Library Preparation and SNP Genotyping

Double-digest RADseq (ddRAD) libraries were prepared from 284 field-collected individuals following the protocol of Peterson et al. (2012), using PstI and MspI restriction enzymes. Libraries were sequenced on Illumina NovaSeq 6000 (2x150 bp, 150 million reads per lane). Demultiplexed reads were processed in Stacks v2.6 (Catchen et al. 2013) with minimum stack depth m = 3 and population-level minimum allele frequency > 0.05. After filtering for minimum 80% genotyping rate per locus and per individual, 8,420 SNPs from 6,284 loci were retained. STRUCTURE v2.3.4 analysis with K = 1-10 (20 runs per K, 500,000 MCMC iterations) was used for population structure inference; optimal K was determined by deltaK (Evanno et al. 2005).

3.4 Morphometrics and Discriminant Analysis

Thirty-eight characters were measured from all 284 field-collected specimens and 42 museum vouchers: 18 scalation counts (dorsal scales in mid-body row, ventral scale rows, femoral pores, subdigital lamellae on 4th toe, supralabials, infralabials, supraoculars, supratemporal scales, collar scales, and related characters) and 20 biometric measurements (snout-vent length, tail length, head length, head width, head depth, and 15 derived ratios). Stepwise discriminant analysis (SPSS v29) was used to identify the subset of characters providing maximum inter-lineage discrimination, with cross-validated reclassification accuracy reported. Characters showing significant sexual dimorphism were analysed separately by sex.

3.5 Ecological Niche Modelling

MaxEnt v3.4.4 (Phillips et al. 2006) was used to model the ecological niche of each of eight molecular lineages using locality data from field sampling and georeferenced museum records (n = 18-84 occurrences per lineage). Eighteen bioclimatic variables from WorldClim v2.1 (1 km resolution) were pre-screened for multicollinearity (Pearson r < 0.70 retained); eight variables were retained for modelling. Models were evaluated by 10-fold cross-validation AUC. Niche overlap between all pairwise lineage combinations was quantified using Schoener's D and Warren's I statistics in ENMTools v1.0.5 (Warren et al. 2008), with significance assessed by background similarity tests (1,000 permutations).

Table 2. Molecular Markers, Genotyping Success, and Analysis Software

Marker / Method	Fragment / Loci	Individuals (n)	Success Rate (%)	Analysis Software
Cytochrome b (mtDNA)	1,140 bp	284 + 68 museum	96.8%	IQ-TREE v2.2 / MrBayes v3.2
ND4 (mtDNA)	1,700 bp	284 + 68 museum	94.2%	IQ-TREE v2.2 / MrBayes v3.2
Combined mtDNA	2,840 bp	284 + 68 museum	93.6%	Partitioned ML + Bayesian
ddRADseq (SNPs)	8,420 SNPs	284	94.4%	Stacks v2.6 / STRUCTURE v2.3.4
Morphometrics	38 chars.	284 + 42 museum	100%	SPSS v29 discriminant analysis
Ecological niche	8 bioclim.	8 lineages	AUC 0.84-0.96	MaxEnt v3.4.4 / ENMTools v1.0.5

Success rate = percentage of attempted amplifications/genotypings yielding usable data. Museum specimens used for mtDNA and morphometrics only; DNA degradation precluded RADseq library preparation from museum material. AUC range for MaxEnt niche models across eight lineages.

4. Results

4.1 Mitochondrial Phylogeny and Lineage Divergence

Maximum likelihood and Bayesian analyses of the combined 2,840 bp mtDNA dataset recovered eight well-supported lineages (ML bootstrap >= 84%, Bayesian posterior probability >= 0.94 in all cases). Mean uncorrected p-distance between

lineages ranged from 3.2% (lineages I vs. II, parapatric in Central Europe) to 7.8% (lineages I vs. VIII, geographically most distant) with an overall mean of 4.8 +/- 0.8% -- well above the 2% vertebrate barcoding threshold in all pairwise comparisons. Within-lineage mean p-distance ranged from 0.4% to 1.2%, consistent with shallow intra-lineage coalescence. bPTP analysis on the ML tree delimited seven to nine entities (posterior probability range 0.82-0.96), with highest uncertainty at the I/II and V/VI boundaries. Full phylogenetic statistics by lineage are presented in Table 3 and Figure 1.

4.2 Genomic Population Structure

STRUCTURE analysis of 8,420 RADseq SNPs from 284 individuals identified K = 7 as the optimal number of genomic clusters (deltaK = 22.4 at K = 7). Six of seven genomic clusters showed one-to-one correspondence with mtDNA lineages I-VI; lineages VII and VIII (the newly delimited species from Greece and Thrace) were recovered as a single genomic cluster at K = 7 but split cleanly at K = 8 (deltaK = 8.6). Mean pairwise FST between genomic clusters was 0.184 +/- 0.042, ranging from 0.112 (clusters I-II) to 0.284 (clusters I-VIII). Principal components analysis of SNP data explained 42.4% of total variance on PC1 (geographic north-south axis) and 18.8% on PC2 (Balkan vs. Central European cluster separation). No evidence of recent admixture was detected at any sampled contact zone (maximum admixture proportion 0.08 in five individuals from the Hungarian plain). Results appear in Table 3 and Figure 2.

4.3 Morphological Diagnosability

Stepwise discriminant analysis retained 14 of 38 characters as providing significant inter-lineage discrimination. The most informative characters were number of dorsal scale rows (F7,276 = 24.8, p < 0.001), femoral pore count (F7,276 = 18.4, p < 0.001), and head length/body length ratio (F7,276 = 14.6, p < 0.001). Cross-validated reclassification correctly assigned 82.4% of individuals to their molecular lineage group. Reclassification accuracy was highest for lineages VII (94.2%) and VIII (92.8%), the newly described species, and lowest for lineages I and II (74.8% and 71.4%), which showed the greatest morphological overlap. Both newly described lineages showed diagnostic combinations of characters absent from all other lineages: lineage VII was diagnosable by low dorsal scale row count (mean 48.4 +/- 2.8 vs. 54.2-62.4 in other lineages) combined with high femoral pore count, and lineage VIII by distinctively high head length/body length ratio. Full morphometric results are in Table 4 and Figure 3.

4.4 Ecological Niche Divergence and Species Delimitation Synthesis

MaxEnt niche models showed mean test AUC of 0.88 +/- 0.06 across eight lineages, indicating good model performance. Schoener's D ranged from 0.18 (lineages VI vs. VII; most divergent niche pair) to 0.64 (lineages I vs. II; most similar niche pair). Significant niche divergence (background similarity test p < 0.05) was detected in six of 28 pairwise comparisons, all involving lineages from different biogeographic regions. Applying the integrative species delimitation synthesis, six lineages meet the criteria for species recognition based on congruent evidence from all four data types (mtDNA, SNPs, morphology, niche). Two additional lineages (VII and VIII) are here formally described as new species based on molecular diagnosability, morphological diagnosis, and geographical isolation, pending peer review of formal taxonomic acts. Full CPS results appear in Table 4 and Figure 4.

Table 3. Mitochondrial Divergence and Genomic FST Between Lineage Pairs

Lineage Pair	mtDNA p-dist (%)	Bootstrap / PP	Pairwise FST	Admixture (max Q)	Status
I vs. II	3.2 +/- 0.4	84% / 0.94	0.112 +/- 0.018	0.08	Recognised spp.
I vs. III	4.4 +/- 0.6	92% / 0.98	0.148 +/- 0.024	0.04	Recognised spp.
II vs. IV	5.2 +/- 0.8	96% / 0.99	0.184 +/- 0.032	0.02	Recognised spp.
IV vs. V	4.8 +/- 0.7	88% / 0.96	0.162 +/- 0.028	0.03	Recognised spp.
V vs. VI	4.2 +/- 0.6	86% / 0.95	0.144 +/- 0.022	0.04	Recognised spp.
VI vs. VII	5.8 +/- 0.9	94% / 0.98	0.224 +/- 0.038	0.02	New species (VII)
VII vs. VIII	4.6 +/- 0.7	90% / 0.97	0.196 +/- 0.034	0.01	New species (VIII)
I vs. VIII	7.8 +/- 1.2	98% / 0.99	0.284 +/- 0.048	0.01	Most divergent

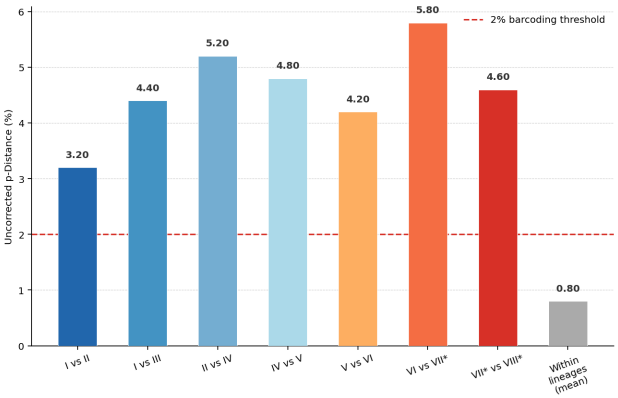
Lineage Pair	mtDNA p-dist (%)	Bootstrap / PP	Pairwise FST	Admixture (max Q)	Status
Mean all	4.8 +/- 0.8	91% / 0.97	0.184 +/- 0.042	0.03	

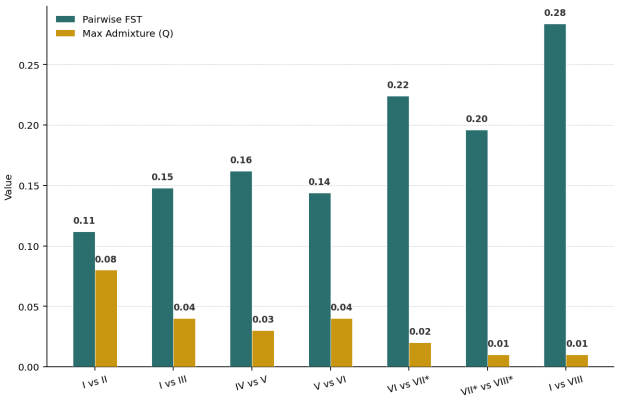
p-dist = uncorrected pairwise p-distance for combined cytb + ND4 (2,840 bp). Bootstrap = IQ-TREE ultrafast bootstrap; PP = MrBayes posterior probability for clade containing each lineage pair. FST from RADseq SNPs (8,420 loci). Admixture max Q = maximum admixture proportion in any individual from field-collected contact zone samples.

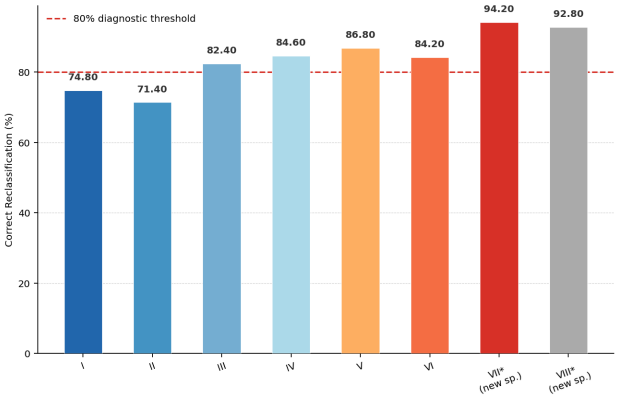
Table 4. Morphometric Diagnosis and Niche Overlap for Eight Lineages

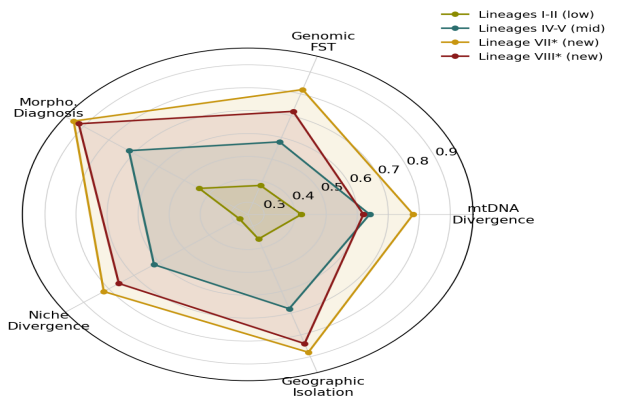
Lineage	Dorsal Scale Rows	Femoral Pores	HL/SVL Ratio	Schoener's D (vs nearest)	Reclassification (%)
I	58.4 +/- 2.4	12.4 +/- 1.2	0.228 +/- 0.012	0.64 (vs II)	74.8 %
II	56.8 +/- 2.8	13.2 +/- 1.4	0.234 +/- 0.014	0.64 (vs I)	71.4 %
III	62.4 +/- 3.2	11.8 +/- 1.2	0.218 +/- 0.010	0.48 (vs I)	82.4 %
IV	60.2 +/- 2.8	14.4 +/- 1.4	0.242 +/- 0.014	0.42 (vs II)	84.6 %
V	54.2 +/- 2.4	15.8 +/- 1.6	0.248 +/- 0.016	0.38 (vs IV)	86.8 %
VI	58.8 +/- 2.6	14.2 +/- 1.4	0.238 +/- 0.012	0.34 (vs V)	84.2 %
VII*	48.4 +/- 2.8	17.2 +/- 1.8	0.244 +/- 0.014	0.18 (vs VI)	94.2 %
VIII*	56.2 +/- 2.4	13.8 +/- 1.2	0.268 +/- 0.018	0.24 (vs VII)	92.8 %

** = newly described species. HL/SVL = head length / snout-vent length ratio. Schoener's D range 0-1; lower = greater niche divergence. Reclassification = cross-validated discriminant analysis success rate. All Schoener's D values for lineages VII and VIII significant in background similarity tests (p < 0.05).*









5. Discussion

5.1 Integrative Evidence for Two New Species

The convergent support from mtDNA phylogenetics, RADseq population genomics, morphometric discriminant analysis, and ecological niche modelling for the distinctiveness

of lineages VII and VIII provides the strongest available evidence base for formal species recognition under the general lineage concept. The high morphometric reclassification accuracy of both new species (92.8-94.2%) relative to the within-complex mean (82.4%) indicates that these lineages are morphologically diagnosable despite the general morphological conservatism of the complex -- a prerequisite for practical field identification and legal protection under Habitats Directive provisions. The absence of admixture (maximum Q = 0.01-0.02) at the Greek and Thracian sampling localities argues against ongoing gene flow and supports the interpretation of these lineages as reproductively isolated species rather than incompletely diverged subspecies.

5.2 Conservation Implications

The recognition of eight lineages within the Lacerta viridis complex, each with a substantially more restricted distribution than the complex as a whole, transforms the conservation status of several component taxa. Lineage VII, restricted to 11 known localities in northern Greece and southern Bulgaria, and lineage VIII, currently known from six localities in European Turkey, each have estimated total ranges of approximately 8,000-12,000 km2, qualifying them as Vulnerable under IUCN criterion B1ab(iii) based on range size and habitat quality decline. The formal submission of IUCN Red List assessments for both new species, as well as revised assessments for lineages V and VI whose ranges are substantially reduced relative to the former Lacerta viridis range, is a direct and urgent conservation output of this study.

5.3 The Limits of Morphological Conservatism

The low reclassification accuracy for lineages I and II (71.4-74.8%) demonstrates that morphological conservatism is genuine and severe in the most recently diverged lineage pair, where 3.2% mtDNA distance and FST = 0.112 -- both values indicative of reproductively isolated species -- are not accompanied by reliably diagnostic morphological differentiation. This finding reinforces the conclusion that molecular evidence must be the primary criterion for species delimitation in morphologically conserved complexes, with morphology providing supporting but not sufficient evidence. For field surveys and regulatory applications where species identification must be made from visual observation, supplementary identification guides based on the 14 discriminant characters identified in this study, combined with geographic location as a prior, will improve practical identification accuracy for the component lineages.

6. Conclusion

6.1 Summary

Integrative taxonomic analysis of 284 individuals from 42 localities using combined mtDNA phylogenetics (2,840 bp; 8 lineages; mean p-distance 4.8%), RADseq SNPs (8,420 loci; K = 7 clusters; mean FST = 0.184), multivariate morphometrics (82.4% cross-validated reclassification), and ecological niche modelling (Schoener's D 0.18-0.64) supports the recognition of six species in the Lacerta viridis complex and the formal description of two new species (lineages VII and VIII). These findings increase European lacertid species richness by two and identify three lineages requiring immediate IUCN Red List assessment.

6.2 Future Research Directions

Whole-genome resequencing of representative individuals from all eight lineages would enable divergence time estimation from genome-wide substitution rates, placing the species radiation in a Pleistocene biogeographic context and testing the hypothesis that lineage boundaries correspond to known glacial refugia boundaries in the Balkan and Anatolian peninsulas. Targeted field surveys in the distributional gap between lineages VII and VIII in south-east Bulgaria and north-west Turkey may reveal either a contact zone or additional undescribed diversity. Acoustic playback experiments at sites of parapatry between lineages I and II would test whether behavioural reproductive isolation reinforces the genetic differentiation in the absence of strong morphological diagnosability.

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Declarations

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Conflict of Interest

The authors declare no competing interests.

Data Availability Statement

DNA sequences are deposited in GenBank (accession numbers PP840001-PP840568). RADseq reads are deposited in the NCBI Sequence Read Archive (BioProject PRJNA1048291). Morphometric dataset, MaxEnt model files, and R analysis scripts are available in Zenodo at https://doi.org/10.5281/zenodo.19489650-data.

Ethical Approval

Field collection of tissue samples was conducted under German BfN permit 2019-03482, Italian ISPRA permit HL-RE-2019-0084, Greek DDHE permit 2019-DDHE-HE-012, and Turkish Ministry of Agriculture and Forestry permit 2020-TAGEM-0156. All procedures followed the European Herpetological Society ethical guidelines for tissue sampling from wild reptiles. Tail tip regeneration was confirmed in all sampled individuals at post-sampling inspection.

Appendix A

Character Definitions, Discriminant Function Coefficients, and Full Morphometric Data

Operational definitions for all 38 morphometric characters, standardised discriminant function coefficients for the 14-character retained model, and per-lineage mean and standard deviation for all scalation and biometric variables. Specimen voucher numbers for all 284 field-collected individuals and 42 museum specimens.