Insect Systematics and Diversity, 2025, **9**(5), ixaf032
DOI: https://doi.org/10.1093/isd/ixaf032
Research

Cryptic diversity on Apennine sky islands: evolutionary history of flea beetles of the *Psylliodes springeri* (Coleoptera, Chrysomelidae) species complex

Emanuele Berrilli*, Maurizio Biondi, Paola D'Alessandro, and Daniele Salvi

Department of Health, Life and Environmental Sciences, University of L'Aquila, L'Aquila, Italy

*Corresponding author. Department of Health, Life and Environmental Sciences, University of L'Aquila, Via Vetoio, 67100 Coppito, L'Aquila, Italy (Email: emanuele.berrilli@univaq.it).

Subject Editor: Adriana Marvaldi

High-altitude environments on isolated mountain peaks harbor unique biodiversity, offering natural laboratories to study past climate change impacts on speciation. In Europe, the Italian Apennines stand out for their high insect endemism, including the micro-endemic flea beetles *Psylliodes springeri* Leonardi, 1975 and the more widely distributed *P. biondii* Leonardi, 1975, which shows a morphologically distinct population on the Maiella Massif. Using species delimitation methods, multispecies coalescent models, and a multilocus molecular approach, we identified key phylogenetic lineages and estimated the timing of cladogenetic events shaping this diversity. Phylogenetic analysis confirmed the monophyly of the *springeri* species complex, consistent with their morphological and ecological similarities. Large genetic distances and lineage sorting in both mitochondrial and nuclear gene trees distinguish the Maiella population as a separate lineage from *P. biondii*. Genetic differentiation between these 2 lineages matches the interspecific distance observed between *P. biondii* and *P. springeri*. Molecular dating places their divergence in a short time frame during the Early Pleistocene, approximately 2 million years ago, likely driven by glacial–interglacial cycles, which isolated populations and triggered divergence. While *P. springeri* and the Maiella lineage remained confined to their respective single massifs, *P. biondii* exhibited a broader distribution, suggesting distinct ecological responses to climate fluctuations. This study underscores how climate-driven isolation has fueled rapid speciation in the Sky Island beetles of the central Apennines, shedding light on the evolutionary history of the largely unexplored biodiversity of high-altitude southern European ecosystems. Future studies may offer further insight into the evolutionary and taxonomic status of the Maiella lineage.

Keywords: Coleoptera, Chrysomelidae

Introduction

High-altitude mountain environments are natural laboratories for studying evolutionary processes shaped by geographic isolation (Love et al. 2023). Species diversification in mountain regions often arises from the interplay of rapid geological and climatic changes associated with orogenic events (Antonelli et al. 2018, Hoorn et al. 2018, Rahbek et al. 2019). These changes typically create environmental discontinuities between once-connected habitats, leading to isolation and allopatric divergence among populations and species (Steinbauer et al. 2016). The fragmentation and isolation in mountainous regions result from the separation of climatically similar habitats by harsh climate zones, which act as dispersal barriers, much like oceans do between islands (Hughes and Eastwood 2006). Consequently, clusters of mountains where environmental conditions at higher elevations differ significantly from those in the surrounding valleys are referred to as "Sky Islands."

The European continent is characterized by a large number of mountain systems, particularly in its southern portion. The geographical heterogeneity shaped by these mountainous areas, combined with the cyclical climatic changes of the Quaternary, has significantly influenced species' evolutionary processes and current distributions (Hewitt 1996, Schmitt 2007, Svenning et al. 2015). The repeated alternation between relatively short warm periods and longer cold periods caused many species to undergo range shifts, resulting in disjunct distribution patterns during at least one of these phases (Taberlet et al. 1998, Hewitt 1999). Throughout these fluctuations, mountains played a crucial role for many cold-adapted alpine species. During interglacial periods, mountains served as refugia, particularly in southern Europe, while in glacial periods they transformed into a complex mosaic of fragmented high-altitude environments (Hughes et al. 2006, Schmitt 2009, Guerrina et al. 2021). These expansion–contraction and fragmentation–isolation processes have left a significant imprint on the genetic diversity and structure of populations inhabiting Sky Islands, making mountains reservoirs of biodiversity. For these reasons, European mountain ranges host a significant proportion of species and are particularly rich in endemic taxa (Kenyeres et al. 2009, Smyčka et al. 2017, Amori et al. 2019).

Received: 25 February 2025; **Revised:** 26 June 2025; **Accepted:** 11 July 2025

Among European mountain systems, the Apennine range remains relatively under-sampled in terms of biodiversity, particularly for many invertebrate groups. As the southernmost mountain system in Europe, it represents, along with the Alps, the main mountain ranges on the Italian peninsula, which served as a key refugium during the glaciations (Taberlet et al. 1998, Hewitt 1999, Habel et al. 2005). The central sector of the Apennines is characterized by its highest peaks, such as the Gran Sasso, which reaches elevations over 2,900 m, and the Maiella, with summits exceeding 2,700 m. The geological history of this area has been profoundly shaped by the Quaternary glaciations (Giraudi and Frezzotti 1997, Giraudi et al. 2004), which moulded both the landscape and species distribution. The high peaks and glacial valleys provided critical refugia for cold-adapted species, enabling them to survive climatic fluctuations. These unique features make this region one of the richest in species diversity on the Italian peninsula (Maiorano et al. 2006, Biondi et al. 2013, Urbani et al. 2015, Menchetti et al. 2021). Furthermore, the Central Apennines present a high rate of endemism when compared with other Mediterranean mountain systems, which further underlines their ecological and evolutionary importance (Conti and Bartolucci 2016, Valle et al. 2022).

Flea beetles of the genus *Psylliodes* Latreille (Coleoptera, Chrysomelidae, Alticini) represent an excellent model for studying the biogeographical significance of the central Apennines. Since the mid-20th century, several new species of *Psylliodes* have been described from the Apennine region (Leonardi 1975, 2007), particularly from its central sector (Leonardi 2007, Biondi and D'Alessandro 2017), underscoring the significant underestimation of the region's biodiversity. Among these, two new species are associated with high-altitude environments in the central Apennines: *Psylliodes springeri* Leonardi, 1975, a micro-endemic species restricted to the Pilato Lake valley in the Sibillini Mountains, and *Psylliodes biondii* Leonardi, 2007, which has a broader distribution encompassing most of the high peaks of the central Apennines, from the Gran Sasso massif to the Matese mountains (Fig. 1; Leonardi 2007). Both species share a similar ecological niche and habitat, living in high-mountain environments above 1,500 m and relying on host plants from the Brassicaceae family, particularly species of the genera *Isatis* and *Erysimum*. Morphologically, they are also closely related to each other and to the Alpine species *Psylliodes picipes* Redtenbacher, 1849 (Leonardi 2007). Despite the morphological similarities within this species complex, high morphological variability has been observed in *P. biondii*. Populations from the northern part of its range differ from those in the southern part. Specifically, individuals from the Maiella massif, although classified as *P. biondii* by Leonardi (2007), were noted by the same author to exhibit morphological peculiarities warranting further investigation. Given the limited migratory and dispersal capacities of these species, attributed to their subapterous or micropterous wings (Leonardi 2007), it is plausible that gene flow among *P. biondii* populations was constrained during warm interglacial phases. Consequently, the effects of isolation in mountainous environments, coupled with the climatic fluctuations experienced by the central Apennines, are likely to be prominently reflected in the morphological variability and genetic structure of this group. Nonetheless, the evolutionary history of this group remains unresolved. It is yet to be determined whether the peculiar traits of the Maiella population represent local phenotypic variation or an independent evolutionary lineage distinct from the widely distributed *P. biondii*.

To investigate the evolutionary history of the *P. springeri* species complex in the Apennine sky islands, we conducted a comprehensive analysis of its phylogenetic structure. The primary objectives of this study are twofold: first, to establish a robust phylogenetic framework for the *P. springeri* species complex and identify its evolutionary and taxonomic units; second, to explore the evolutionary processes driving their diversification within the biogeographic context of the high-mountain Apennine biota. Using a multispecies coalescent model, we aim to estimate divergence times of cladogenetic events within the group to assess whether speciation events correspond to isolation on distinct mountain ranges during Quaternary glacial cycles. Beyond elucidating the evolutionary history of this species complex, this approach will also provide insights into the biogeographic processes that have shaped the high-altitude biotas of the central Apennines.

Methods

Study System

Psylliodes biondii shows pronounced intraspecific morphological and chromatic variation. Northern populations (Abruzzo, locality: 4 to 6, 12) typically display weak metallic blueish reflections especially on the pronotum, more rarely greenish or golden; pronotal surface is generally weakly microgranulate and may appear glossy when microgranulation is absent; elytral surface is relatively smooth, with very faint or absent microgranulation and interstrial punctulation on the disc very fine but distinct, particularly in populations from the Gran Sasso massif. In contrast, southern populations (Molise, locality: 13) normally lack metallic reflections on both the pronotum and the elytra; pronotal surface is less glossy and shows more evident microgranulation compared with the northern populations; elytral surface tends to be smoother with the interstrial punctation barely visible or entirely absent. The specimens from the Maiella massif show an average elytral length of 2.16 mm, which is markedly greater than the species average. As no females were available for this population at the time of *P. biondii* description, Leonardi (2007) preferred to exclude the specimens from Maiella from the type series. Compared with *P. springeri*, *P. biondii* has habitus generally more convex and oval-shaped, with clearly weaker metallic reflections, and darker legs. The aedeagus is generally more slender; however, the most reliable diagnostic characters distinguishing the two species are the spermatheca, with a longer ductus in *P. biondii*, and the shape of the vaginal palpi, with a larger and not angled proximal part in *P. biondii* (Leonardi 2007).

Sample Collection and Morphological Identification

For this study, 34 specimens of *P. biondii* Leonardi, 2007 and 45 specimens of *P. springeri* Leonardi, 1975 were collected from nine localities in central Apennine (Fig. 1; Table 1). Specimens were collected on *Isatis apennina* and *Erysimum pseudorhaeticum* by aspirator and then stored in 95% ethanol. Morphological identification at the species level was performed by Maurizio Biondi through the dissection and study of the genitalia, median lobe of the aedeagus for males and

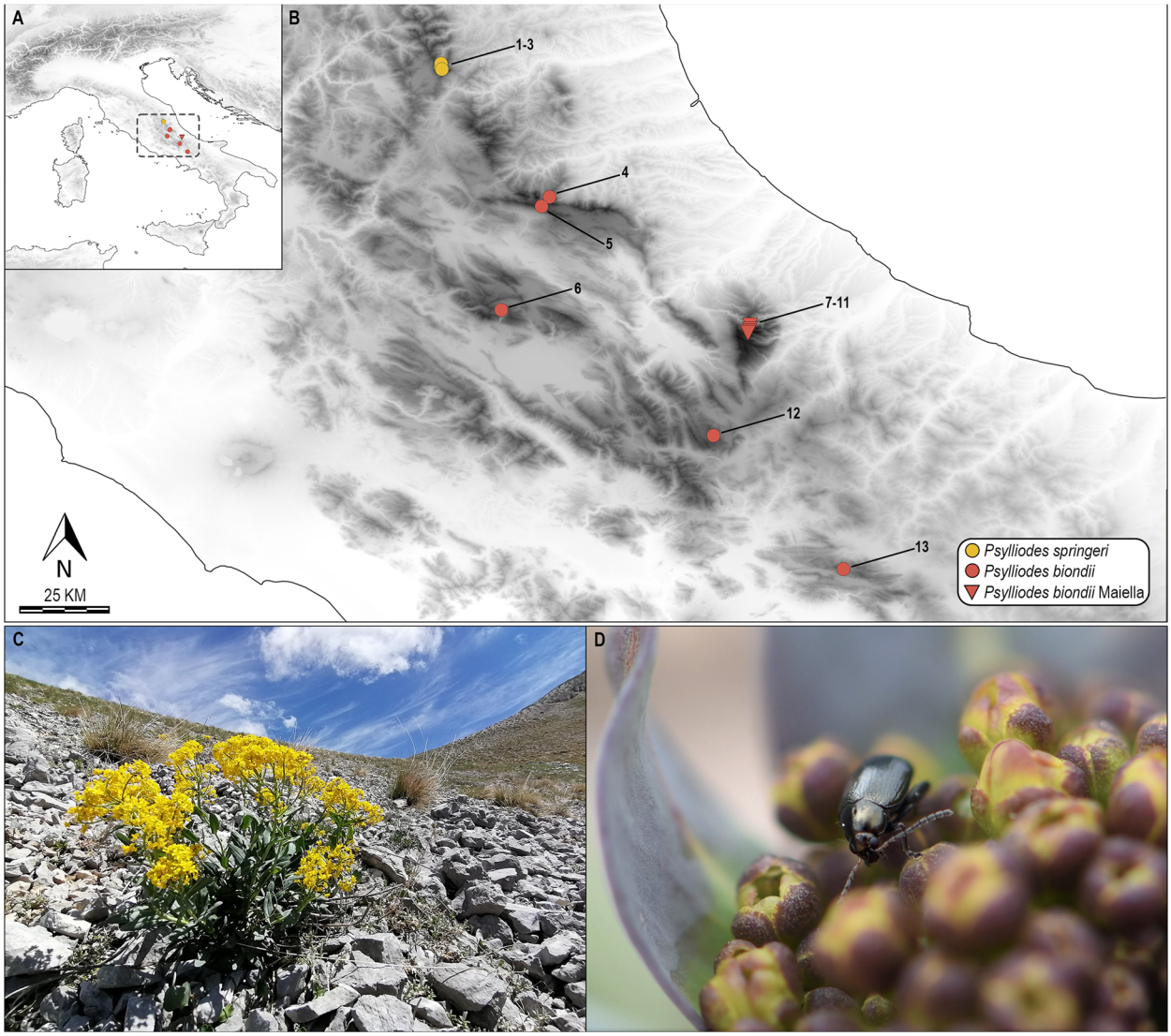

Fig. 1. A) Map of Italy with the study area highlighted (dashed rectangle); B) Close-up of the central Apennines region with sampling localities of *P. springeri* (yellow) and *P. biondii* (red); C) The host plants *Isatis apennina* from Velino mountain, in a typical environment for species of the *springeri* species complex (photo by E. Berrilli); D) Individual of *P. biondii* of the Maiella population feeding on an inflorescence of *I. apennina* (photo by E. Berrilli).

spermatheca for females. Dissection of the specimens was carried out using a Leica M205C binocular microscope.

DNA Extraction, Amplification, and Sequencing

Total genomic DNA was extracted following a standard high-salt protocol (Sambrook et al. 1989), using the noninvasive approach described by Salvi et al. (2020). Five fragments from four genes, including both mitochondrial and nuclear genes, were amplified via polymerase chain reaction (PCR). Two fragments of the cytochrome oxidase subunit I (*cox1*) gene were targeted: (i) the standard barcode region was amplified using universal primers LCO1490 and HC02198 (Folmer et al. 1994), and (ii) the 3′ fragment using primers C1-J-2183 and TL2-N-3014 (Simon et al. 1994). Amplification conditions for the mitochondrial fragments followed those detailed in Salvi et al. (2019). Three protein-coding single copy nuclear genes were amplified from a random subset of individuals within each population: Carbamoylphosphate synthase (*CAD*), Crossveinless 2 (*Cv2*), and Rad 50 protein (*Rad50*). The nuclear markers were amplified using primers and amplification conditions described in Berrilli et al. (2023). Successful amplification was determined by gel electrophoresis, and PCR products were purified and sequenced by an external service (Genewitz, United Kingdom). The obtained chromatograms of each sequence were manually edited and assembled into a consensus sequence using Geneious Prime 2021 (Biomatters Ltd, Auckland, New Zealand).

Species Delimitation and Phylogenetic Analyses

Sequences of each gene were aligned separately using MAFFT v7.450 with the G-INS-I progressive method algorithm (Katoh et al. 2002) and then used for downstream phylogenetic analysis. First, we performed species delimitation (SD) analysis using the standard barcode fragment of *cox1* to infer putative species boundaries within the *springeri* species complex.

Table 1. Details on sampling localities for each studied species. Locality ID represents the locality number reported in the map of Fig. 1

Species	Locality ID	Locality	N° specimens	Elevation (m)	Coordinates
Psylliodes springeri	1	Pilate Lake Valley	12	1,788	42.83, 13.26
	2	Pilate Lake Valley	10	1,576	42.84, 13.26
	3	Pilato Lake	23	1,958	42.83, 13.27
Psylliodes biondii	4	Sella dei due Corni	3	2,517	42.47, 13.56
	5	Gran Sasso, Passo della Portella	2	2,246	42.45, 13.54
	6	Sirente Mountain, Pizzo Trento	4	2,054	42.16, 13.43
	7	Maiella, Focalone Mountain	4	2,639	42.1, 14.11
	8	Maiella, Focalone Mountain	1	2,167	42.12, 14.12
	9	Maiella, Focalone Mountain	2	2,624	42.11, 14.11
	10	Maiella, Focalone Mountain	1	2,657	42.1, 14.11
	11	Maiella, Focalone Mountain	12	2,586	42.1, 14.11
	12	Aremogna Plateau	3	2,111	41.82, 14.01
	13	Matese, Miletto Mountain	2	2,047	41.45, 14.37

We performed three SD analyses to infer the number of putative species and their correspondence to morphospecies: (i) nucleotide distance with thresholds estimated ad hoc on the data set, (ii) Assemble Species by Automatic Partitioning (ASAP; Puillandre et al. 2021) and (iii) multirate Poisson Tree Processes model (mPTP; https://github.com/Pas-Kapli/mptp; (Kapli et al. 2017)). The pairwise nucleotide distance matrices required for ad hoc nucleotide distance threshold methods were estimated using the R library *ape* v5.3 (Paradis and Schliep 2019). Intraspecific and interspecific genetic distances were calculated based on the Kimura 2-Parameter (K2P) substitution model with the pairwise deletion option. A threshold optimization analysis was performed with the R package *spider* v1.5.0 (Brown et al. 2012) and the best threshold was identified with the *localMinima* function. Finally, function *tclust* of the package *spider* was used to cluster nucleotide sequences at the *ad hoc* threshold value previously identified. ASAP analyses were run using the program web-interface (https://bioinfo.mnhn.fr/abi/public/asap); K2P was selected as nucleotide substitution model and other parameters were left as default (Puillandre et al. 2021). For the mPTP analysis, we first generated a Maximum likelihood (ML) tree based on *cox1* sequences in IQ-TREE 1.6.12 (Nguyen et al. 2015) using the W-IQ-TREE webserver (Trifinopoulos et al. 2016). The best substitution models for *cox1* alignment were determined by the ModelFinder module, including flexible rate heterogeneity across sites models (Kalyaanamoorthy et al. 2017), based on the Bayesian Information Criterion. Branch support was assessed by 1,000 replicates of ultrafast bootstrapping (uBS) (Minh et al. 2013, Hoang et al. 2018). The phylogenetic tree was used as input of the mPTP analysis, which was performed in mPTP v0.2.4 using 10 runs of 100 million MCMC generations each, sampling every 10,000 (burn-in = 10%). The convergence of the independent runs was assessed through the average standard deviation of delimitation support values (ASDDSV) and the overall support for the ML estimate, calculated by computing the mean of the average support values (ASV) over the 10 runs.

To assess whether the major lineages of the *springeri* species complex, identified through SD analysis, form a monophyletic clade, in accordance with morphological characteristics, we constructed a dataset incorporating sequences from the two *cox1* fragments and the three nuclear markers (*CAD*, *Cv2*, and *Rad50*) from 63 *Psylliodes* species, sourced from Gikonyo et al. (2024), which currently provides the most comprehensive dataset for *Psylliodes*. As outgroup taxa, we included *Phyllotreta striolata* (Fabricius) and *P. armoraciae* (Koch) based on their phylogenetic relationships (Gikonyo et al. 2024). We then generated a multilocus ML tree using IQ-TREE, employing the same settings previously used for the ML analysis based on *cox1* sequences, with the addition of the Edge-Linked partition model to allow each partition to have its own evolutionary rate. FigTree v1.3.1 (Rambaut and Drummond 2009) was used to depict the trees. Nodes with uBS values between 80% and 95% were considered moderately supported, whereas uBS values >95% were considered strongly supported.

To assess haplotypic sharing between species or lineages at the level of the three nuclear markers, we used a gene genealogy network approach. Haplotype phase of nuclear genes was determined using DnaSP v5 (Librado and Rozas 2009) under the PHASE algorithm (Stephens et al. 2001, Stephens and Donnelly 2003), with the initial 1,000 iterations discarded as burn-in, 1 as thinning interval and 1,000 post-burn-in iterations. Phylogenetic relationships among haplotypes were inferred through the median-joining distance method (Bandelt et al. 1999) using the PopArt 1.7 software (Leigh and Bryant 2015).

Following the assessment of haplotype relationships and potential sharing among lineages, we subsequently conducted Bayesian species delimitation analyses to formally test species boundaries based on the phased dataset of the three nuclear loci. We performed multilocus Bayesian species delimitation using BP&P v2.2 (Rannala and Yang 2003, Yang and Rannala 2010) to test species boundaries among groups defined by mtDNA data. The model assumes no admixture following speciation, which is an assumption motivated by the biological species concept and a reversible-jump Markov Chain Monte Carlo (rjMCMC) to estimate the posterior distribution for species delimitation models. Both algorithm0 and algorithm1 were run ($\varepsilon = 15$ and $\varepsilon = 2$, respectively) to ensure proper mixing and consistent results (Yang and Rannala 2010). Each species delimitation model was assigned equal probabilities for rooted trees (speciesmodelprior = 1). The rjMCMC analysis was run for 200,000 generations with a sampling interval of 1 and a burn-in of 20,000 (10%). The guide tree was based on the multilocus ML tree, and speciation probabilities were summarized across models, considering ≥95% as strong support (Leaché and Fujita 2010). To evaluate the effect of priors on posterior probability (PP), we tested three combinations of prior settings for these parameters (Leaché and Fujita 2010),

which are given as gamma G(α, β) distributions: relatively large ancestral population size and shallow divergences (θ = *G*[1,10], τ = *G*[2,2,000]), relatively large ancestral population size and deep divergences (θ = *G*[1,10], τ = *G*[1,10]), and relatively small ancestral population size and shallow divergences (θ = *G*[2,2,000], τ = *G*[2,2,000]). The first combination is a conservative one, favoring models containing fewer species. Only speciation events supported by all models were accepted. All analyses were run twice to confirm consistency between runs.

To estimate the species tree and divergence times of the *springeri* species complex in Central Apennine we used the multispecies coalescent method implemented in the StarBeast2 packages of BEAST2 v.2.7.1 (Ogilvie et al. 2017, Bouckaert et al. 2019). For this analysis, we used alignments of the two mitochondrial fragments of *cox1* and phased alignments of the three nuclear genes. We linked the trees of the two mitochondrial fragments and unlinked substitution models and clock models of gene partitions. We used a relaxed clock model (uncorrelated log-normal clock) calibrated using the available substitution rate for the 3′ fragment of *cox1* (clock.rate = 0.0168, SD = 0.062) estimated for beetles by Papadopoulou et al. (2010). We adopted this substitution rate because it has been extensively validated and it is consistent with the average substitution rate traditionally used for mtDNA evolution in Chrysomelidae (eg Montelongo and Gómez-Zurita 2014, Platania et al. 2024), thus providing a reliable, lineage-specific reference. This approach offers a more coherent framework for comparative analyses than calibrations based on unrelated fossil records or secondary calibrations derived from different datasets. The remaining settings were as follows: (unlinked) models of nucleotide substitution for each gene partition with the HKY+G substitution model, with 4 gamma categories, frequencies to empirical and unchecked estimate box of substitution rate, for all the gene partitions; clock rate estimate for each partition; constant population model and a Yule process as species tree prior. StarBeast2 was run two times, with 500 million generations, sampling every 50,000 generations. We used Tracer v.1.7.1 (Rambaut et al. 2018) to check the runs for convergence (burn-in = 25%), LogCombiner and TreeAnnotator to combine runs and summarize the trees in a Maximum Clade Credibility Tree representing the posterior distribution. We used DensiTree and FigTree to visualize the results (Rambaut and Drummond 2009, Bouckaert 2010). Nodes with Bayesian PP values between 0.95 and 0.98 were considered moderately supported, while those PP >0.98 were considered strongly supported.

Results

Molecular Species Delimitation and Mitochondrial Lineages

We obtained 79 sequences for both the two fragments of *cox1* (the standard barcode region of 608 bp and the 3′ fragment of 767 bp), 45 sequences for *P. springeri*, 15 sequences for *P. biondii*, and 20 for the Maiella's population.

ML analyses based on the standard *cox1* barcode fragment identified three main lineages, supporting the separation into two distinct lineages of *P. springeri* and *P. biondi* (uBS = 99; Fig. 2B), as well as the separation of Maiella specimens from the latter (uBS = 99; Fig. 2B). Results of species delimitation analyses using *tclust*, ASAP and mPTP, are consistent with the clusters identified by the ML tree. Specifically, both the highest ASAP score (1.00) and the *tclust* analysis with the optimal distance threshold (3%) consistently identify the three lineages of the *springeri* species as three clusters, which are also supported by the mPTP analysis (run convergence: ASDDSV <0.01) as distinct species clusters (ASV median: 83%) (Fig. 2B).

Multilocus ML analyses based on the mitochondrial and nuclear gene fragments resolved the phylogenetic position of *P. springeri*, *P. biondii*, and the Maiella lineage in a monophyletic group (uBS = 100) within the genus *Psylliodes* (Fig. 2A). Phylogenetic relationships within the *springeri* species complex show *P. springeri* as sister to the clade composed of *P. biondii* and the Maiella lineage (uBS = 100; Fig. 2A).

Median-joining networks based on 46 sequences of *CAD* (822 bp), 42 of *Cv2* (606 bp), and 32 of *Rad50* (687 bp) data show three distinct haplogroups corresponding to *P. springeri*,

P. biondii, and the Maiella lineage (Fig. 3). Phylogenetic networks show a lack of haplotype sharing between the three lineages at any nuclear marker. All three nuclear markers separate the haplotypes belonging to the three lineages by at least eight mutational steps.

Bayesian species delimitation analyses consistently support the three lineages under both the 95% probability threshold and the cumulative criterion. Speciation probabilities were equal to one across all runs, irrespective of the prior settings for ancestral population size (θ) and root age (τ).

The species tree is consistent with the multilocus ML tree based on concatenated gene matrices (Fig. 4A); however, relationships among *Psylliodes springeri*, *P. biondii*, and the Maiella lineage are poorly supported (Fig. 4A and C). Diversification within this species complex occurred during the Early Pleistocene, approximately within the same time frame for the split of *P. springeri* (2.16 million years ago [Mya]; 95% high posterior density interval, HPD95: 2.87 to 1.49 Mya) and the divergence between *P. biondii* and the Maiella lineage (1.95 Mya; HPD95: 2.53 to 1.08 Mya).

Discussion

In this study, using a phylogenetic approach, we uncover previously overlooked diversity within the *P. springeri* species complex in the Central Apennines, identifying the populations from the Maiella Mountains as a distinct evolutionary unit. We link the diversification processes within this group to major environmental and climatic changes during the Early Pleistocene. This highlights how the high peaks of the Apennines, with their complex topography, have both facilitated lineage diversification through a sky island dynamic during periods of climatic changes and provided stable habitats that ensured the persistence of these lineages.

Species Boundaries Within the springeri Complex

Phylogenetic analyses strongly support the monophyly of the *springeri* species complex, and clarify its phylogenetic placement within the Clade A of the genus *Psylliodes* (*sensu* Gikonyo et al. 2024), which originated approximately 16 Mya and includes species primarily associated with Brassicaceae host plants. This finding is in agreement with morphological data and with key ecological traits shared by species of the *springeri* complex. Within the species complex, phylogenetic and species delimitation analyses clearly indicate that the Maiella population is not conspecific of *P. biondii* as genetic differentiation

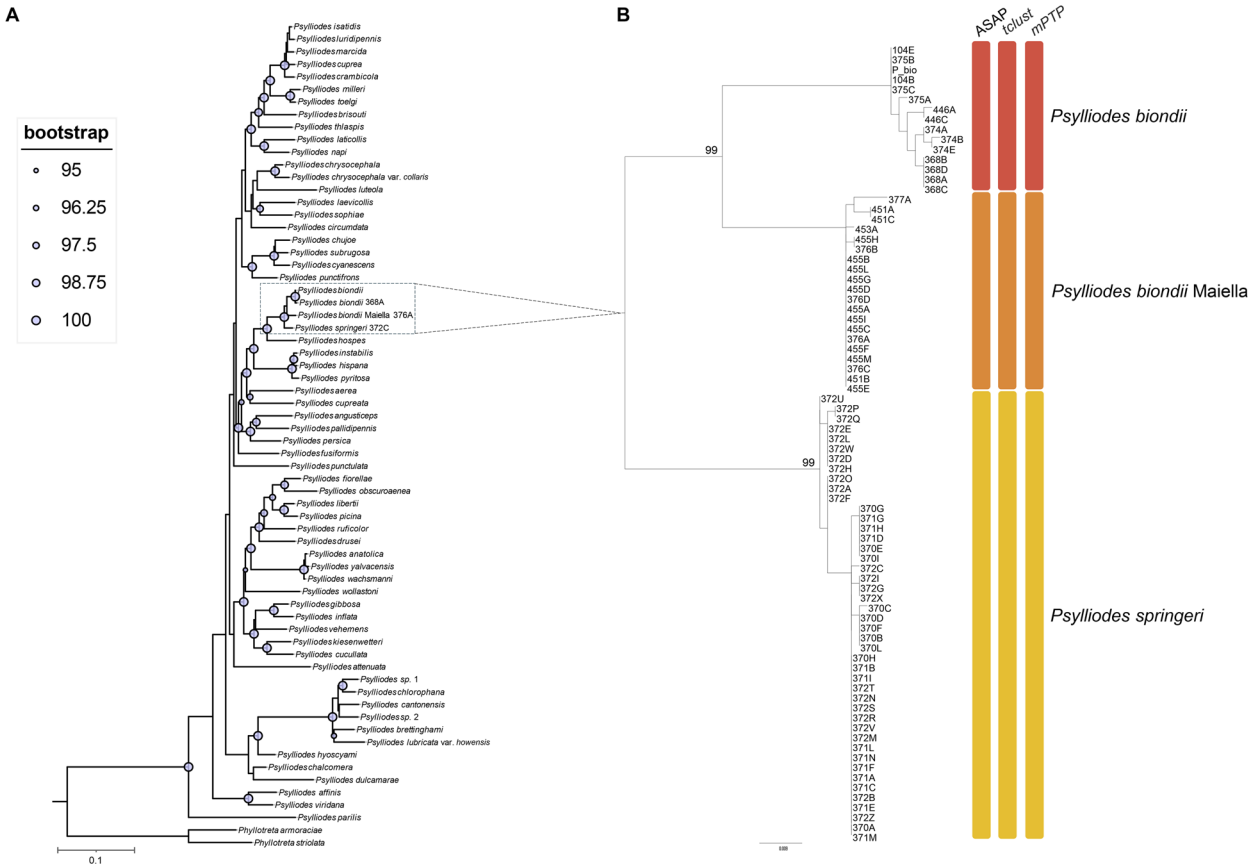

Fig. 2. A) Maximum likelihood phylogenetic tree of *Psylliodes* species based on 2 gene fragments of *cox1* and 3 nuclear gene fragments, *CAD, Cv2,* and *Rad50*. Circles in correspondence with nodes represent uBS. The *springeri* species complex is highlighted by a dashed rectangle. B) Maximum likelihood phylogenetic tree of the *springeri* species complex based on the standard barcode fragment of *cox1*. The bootstrap support is reported on the nodes. For each sequence, the voucher code is reported. Vertical bars on the right of the tree show results of the species delimitation analyses (ASAP, *tclust*, and mPTP).

between these two lineages at mitochondrial loci is similar to the interspecific distance observed between *P. biondii* and *P. springeri* and consistent with values reported for interspecific comparisons within Alticini (Magoga et al. 2018, Salvi et al. 2020) or, more generally, within Chrysomelidae (Magoga et al. 2021). In addition to mitochondrial divergence, there is clear differentiation in nuclear genealogies, where we observed complete lineage sorting among *P. springeri*, *P. biondii*, and the Maiella lineage across all three nuclear markers tested (Fig. 3). In other species of Alticini inhabiting mountain environments, these same markers (*CAD* and *Cv2*) have shown lower levels of divergence or haplotypic sharing, even in cases of allopatric distributions over geographic distances far exceeding those in the *springeri* species complex (Berrilli et al. 2024). The molecular approach used, combining phylogenetic and species delimitation analyses, allowed us to refine species boundaries, compensating for the lack of strongly diagnostic morphological traits needed to reliably distinguish these species. Indeed, classical morphology and morphometric analyses reveal that most traits display considerable variability and overlap within the *springeri* complex, complicating species delimitation (Leonardi 2007). This is the reason why Leonardi classified the Maiella population as *P. biondii*, despite its distinctive morphological traits leading him to exclude this population from the type series (Leonardi 2007). Multiple evolutionary processes may have contributed to the extensive morphological variability observed in populations inhabiting mountain environments, complicating the distinction between intra- and inter-specific variation. A combination of genetic drift, founder events, and local environmental variability likely influences both phenotypic plasticity and local adaptations, driving the rapid evolution of intraspecific morphological diversity or convergent adaptation across various traits (Price et al. 2003, Storz et al. 2010, Mata et al. 2022, Chantepie et al. 2024).

Evolutionary History of the springeri Complex

The observed phylogenetic pattern and geographic structure with complete sorting of the three lineages of the *springeri* species complex, at both fast (mitochondrial) and slow (nuclear) genes, suggest that they have experienced an ancient diversification due to prolonged reproductive isolation in distinct mountain ranges. Speciation events within this complex appear to have occurred in relatively rapid succession during the Early Pleistocene, with both the diversification of *P. springeri* and the split between *P. biondii* and the Maiella lineage taking place approximately 2 Mya. These nearly simultaneous cladogenetic events provide a plausible explanation for the unresolved polytomous branching pattern observed within the species complex. The low posterior probability associated with alternative topologies underlying different relationships among the three lineages (Fig. 4) likely reflects the challenges of resolving the temporal succession of two cladogenetic events that arose over

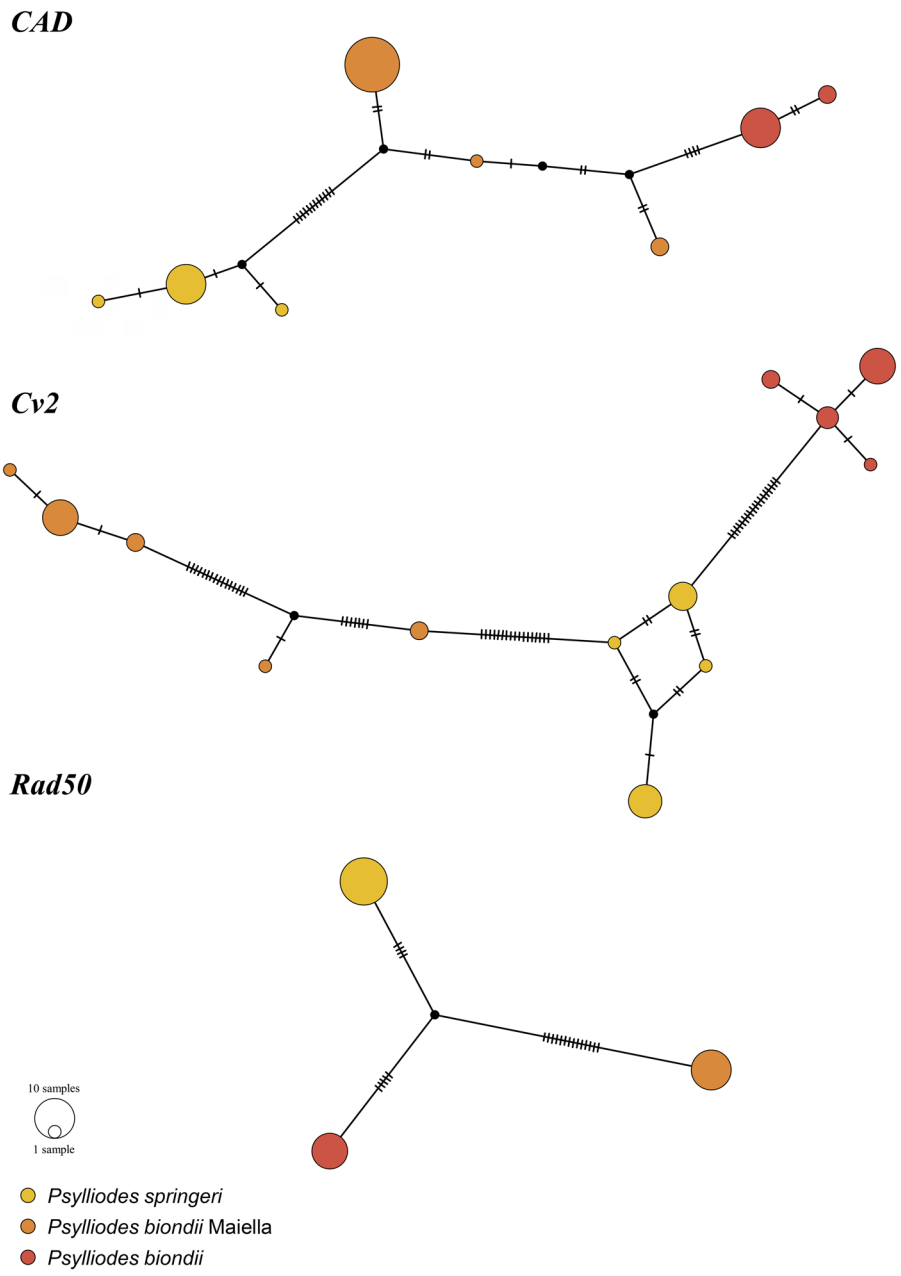

Fig. 3. Haplotype networks showing the phylogenetic relationships within the *springeri* species complex based on 3 nuclear (*CAD, Cv2,* and *Rad50*) markers. Haplotypes are represented by circles colored according to species and lineages, with size proportional to their frequency (see the size reference inset in the bottom left corner). Vertical bars represent mutational steps (nucleotide substitutions).

a short evolutionary timeframe. This scenario aligns with vicariant events that lead to isolation and divergence of multiple populations at once, such as the divergence driven by major glacial–interglacial cycles during Pleistocene (Kahlke et al. 2011). Rapid diversification events in high-altitude mountain habitats during the Pleistocene are well-documented phenomenon across various regions of the world (Linder 2008, Hughes and Atchison 2015, Nevado et al. 2018). Such processes are often attributed to increased ecological opportunities resulting from the pronounced physiographic heterogeneity of mountainous landscapes, which fosters frequent episodes of geographic isolation and allopatric speciation (Simpson 1964, Hewitt 2004, Hughes and Eastwood 2006). Allopatric divergence among lineages endemic to distinct mountain ranges, driven by Pleistocene climate changes, has been suggested for many insects inhabiting high-altitude environments of the Alps and Apennines, such as leaf beetles of the genus *Oreina* (Borer et al. 2010) or several Alpine butterflies (Haubrich and Schmitt 2007, Huemer and Hebert 2011). Specifically, the general cooling of global climatic conditions during early glaciations likely facilitated the downward expansion of suitable habitats for cryophilic species to lower elevations and latitudes, mirroring the widespread distribution patterns observed for many cold-adapted taxa in the early Pleistocene. In contrast, subsequent interglacial warming phases likely led to the fragmentation of these expanded ranges, confining populations to smaller, isolated refugia in mountains area and promoting their genetic differentiation through prolonged isolation (Bertini 2010,

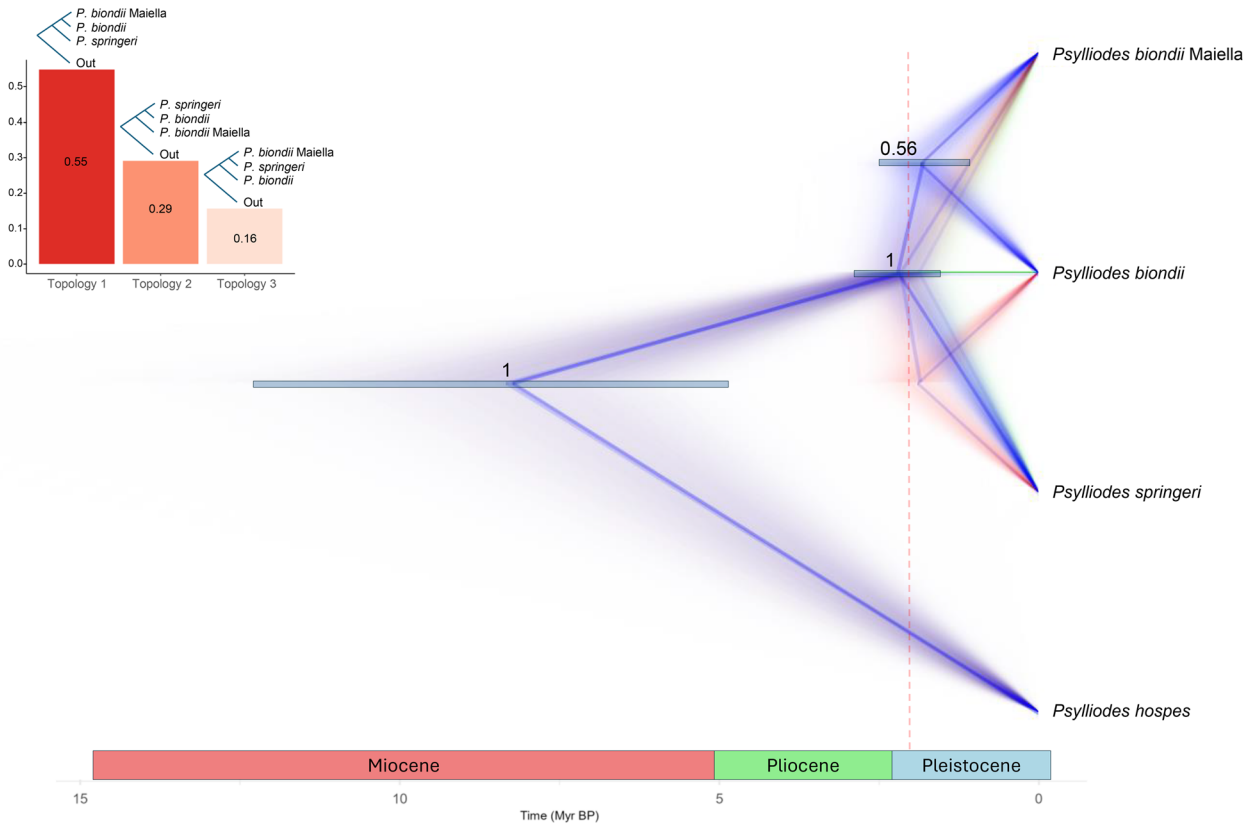

Fig. 4. Time-calibrated species tree of the *springeri* species complex estimated in BEAST showing the consensus tree topology (dark blue line) and trees from the posterior distribution visualized using the software DensiTree. Bayesian posterior probability values are reported on each node along with light blue bars representing the 95% high posterior density interval (HPD95) of node age. Time scale is in millions of years (Ma). The dashed line highlights the 2-million-year mark. The top left histogram shows the proportion of tree topologies supporting alternative relationships among *P. biondii, P. springeri,* and the Maiella lineage.

Chiocchio et al. 2017, Bertini and Combourieu-Nebout 2023). Lineages within the *springeri* species complex, which are ecologically strongly associated with high-altitude environments, and characterized by high fidelity to their host plants and limited dispersal ability, may have experienced isolation and diversification during interglacial phases. However, unlike *P. springeri* and the Maiella lineage, which are each restricted to a single mountain range, *P. biondii* shows a more continuous distribution along the central Apennine, spanning from the Gran Sasso to the Matese mountain ranges. This suggests a distinct ecological response and refugial structure of these species during glacial–interglacial periods. The isolation of *P. springeri* and the Maiella lineages, while intensified during interglacial periods, likely persisted through glacial phases due to their limited range shifts or the presence of unfavorable lowland environmental conditions during glaciations. These factors would have hindered large-scale colonization, ultimately confining these lineages to a single mountain peak. On the other hand, *P. biondii* likely experienced greater habitat and range continuity throughout the glacial periods. Its current distribution is thus interpreted as the result of the fragmentation of a once-continuous range into isolated mountains during the last interglacial period. The persistence of either a single or multiple massifs underlying a scenario of a single refugium or multiple refugia throughout the glacial–interglacial cycles is a well-documented pattern in the Italian fauna. For example, a scenario with relatively connected refugial areas during glacial periods has been suggested for several endemic amphibians and reptiles (Canestrelli et al. 2008, 2012, Salvi et al. 2013, Chiocchio et al. 2021). In contrast, *P. springeri* and the Maiella lineage remained isolated on the Sibillini and Maiella massifs, respectively. The isolation of these two massifs is not unexpected, as both are known to harbor numerous endemic species or taxa that differ from the faunal and floral diversity observed in the rest of the central Apennines (Farina and Leonardi 2018, Conti et al. 2019). However, gaining a deeper understanding of the evolutionary and biogeographic history of this high-altitude group will require comparative studies with closely related species in the Alps, such as *P. picipes*. This comparison could also offer valuable insights into resolving the taxonomic status of the Maiella lineage.

This study highlights how climate-driven isolation processes have shaped speciation events and leaf beetles' diversity of a Sky Island system in the central Apennines. While much of our current understanding of biogeographic responses to Pleistocene climate change stems from research on temperate species, the evolutionary history of mountain taxa in southern European peninsulas remains understudied. Compounding this gap is the great, yet largely unexplored, biodiversity of high-altitude environments. Bridging these knowledge gaps is essential for advancing our understanding of the genetic structure and evolutionary dynamics of mountain species, a critical step toward informing and prioritizing conservation strategies in these fragile and climate-vulnerable ecosystems.

Acknowledgements

We would like to thank Valter di Cecco, Lorenzo De Vitis, Michele Di Musciano, Matteo Garzia, and Giulia Simbula for their help in sample collection. Fieldwork for this project was supported by the PhD Program of the University of L'Aquila, by the Percy Sladen Memorial Fund (granted to EB in 2023), and by the "Progetto di Ricerca di Ateneo per la Ricerca di base e Avvio alla Ricerca" funded by the University of L'Aquila (granted to EB in 2024) and by European Union—Next Generation EU. National Biodiversity Future Center (NBFC)—Spoke 3 "Assessing and monitoring terrestrial and freshwater biodiversity and its evolution: from taxonomy to genomics and citizen science"—CN00000033—CUP B63C22000650007. This work is part of the PhD thesis of Emanuele Berrilli ("Health and Environmental Sciences" PhD Program, University of L'Aquila).

Author Contributions

Emanuele Berrilli (Conceptualization [equal], Data curation [lead], Formal analysis [lead], Funding acquisition [equal], Writing—original draft [lead]), Maurizio Biondi (Data curation [supporting], Funding acquisition [equal], Writing—review & editing [equal]), Paola D'Alessandro (Funding acquisition [equal], Writing—review & editing [equal]), and Daniele Salvi (Conceptualization [equal], Data curation [supporting], Formal analysis [supporting], Funding acquisition [equal], Supervision [lead], Writing—original draft [supporting])

Funding

This project was supported by the PhD Program of the University of L'Aquila, by the Percy Sladen Memorial Fund (granted to EB in 2023), by the "Progetto di Ricerca di Ateneo per la Ricerca di base e Avvio alla Ricerca" funded by the University of L'Aquila (granted to EB in 2024) and by European Union—Next Generation EU. National Biodiversity Future Center (NBFC)—Spoke 3 "Assessing and monitoring terrestrial and freshwater biodiversity and its evolution: from taxonomy to genomics and citizen science"—CN00000033—CUP B63C22000650007.

Conflicts of Interest

None declared.

References

Amori G, Boitani L, Milana G, et al. 2019. Endemism and diversity in European montane mammals: macro-ecological patterns. *Biol. J. Linn. Soc.* 128:225–237.

Antonelli A, Kissling WD, Flantua SG, et al. 2018. Geological and climatic influences on mountain biodiversity. *Nat. Geosci.* 11:718–725.

Bandelt H-J, Forster P, Röhl A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* 16:37–48.

Berrilli E, Biondi M, D'Alessandro P, et al. 2023. Cryptic, sibling or neither of the two? Integrative species delimitation of *Psylliodes* flea beetles with overlapping ranges. *Zool. Scr.* 52:235–248. https://doi.org/10.1111/zsc.12574

Berrilli E, Biondi M, Garzia M, et al. 2024. Apennine–Pyrenees disjunct distribution: an unusual biogeographic pattern revealed in flea beetles of the *Longitarsus candidulus* species-group (Coleoptera, Chrysomelidae). *Curr. Zool.* 70:668–677. https://doi.org/10.1093/cz/zoad050

Bertini A. 2010. Pliocene to Pleistocene palynoflora and vegetation in Italy: state of the art. *Quat. Int.* 225:5–24.

Bertini A, Combourieu-Nebout N. 2023. Piacenzian to late Pleistocene flora and vegetation in Italy: a moving sketch. *Alp. Mediterr. Quat.* 36:91–119.

Biondi M, D'Alessandro P. 2017. *Psylliodes urbaniae*: a new species from Central Apennines (Coleoptera: Chrysomelidae: Galerucinae: Alticini). *Onychium* (13):121–130.

Biondi M, Urbani F, D'Alessandro P. 2013. Endemism patterns in the Italian leaf beetle fauna (Coleoptera, Chrysomelidae). *Zookeys.* 332:177–205. https://doi.org/10.3897/zookeys.332.5339

Borer M, Alvarez N, Buerki S, et al. 2010. The phylogeography of an alpine leaf beetle: divergence within Oreina elongata spans several ice ages. *Mol. Phylogenet. Evol.* 57:703–709.

Bouckaert R, Vaughan TG, Barido-Sottani J, et al. 2019. BEAST 2.5: an advanced software platform for Bayesian evolutionary analysis. *PLoS Comput. Biol.* 15:e1006650.

Bouckaert RR. 2010. DensiTree: making sense of sets of phylogenetic trees. *Bioinformatics* 26:1372–1373.

Brown SD, Collins RA, Boyer S, et al. 2012. Spider: an R package for the analysis of species identity and evolution, with particular reference to DNA barcoding. *Mol. Ecol. Resour.* 12:562–565.

Canestrelli D, Cimmaruta R, Nascetti G. 2008. Population genetic structure and diversity of the Apennine endemic stream frog, *Rana italica*—insights on the Pleistocene evolutionary history of the Italian peninsular biota. *Mol. Ecol.* 17:3856–3872. https://doi.org/10.1111/j.1365-294X.2008.03870.x

Canestrelli D, Salvi D, Maura M, et al. 2012. One species, three Pleistocene evolutionary histories: phylogeography of the Italian crested newt, *Triturus carnifex*. *PLoS One*. 7:e41754. https://doi.org/10.1371/journal.pone.0041754

Chantepie S, Charmantier A, Delahaie B, et al. 2024. Divergence in evolutionary potential of life history traits among wild populations is predicted by differences in climatic conditions. *Evol. Lett.* 8:29–42. https://doi.org/10.1093/evlett/qrad067

Chiocchio A, Arntzen JW, Martínez-Solano I, et al. 2021. Reconstructing hotspots of genetic diversity from glacial refugia and subsequent dispersal in Italian common toads (*Bufo bufo*). *Sci. Rep.* 11:260. https://doi.org/10.1038/s41598-020-79046-y

Chiocchio A, Bisconti R, Zampiglia M, et al. 2017. Quaternary history, population genetic structure and diversity of the cold-adapted Alpine newt *Ichthyosaura alpestris* in peninsular Italy. *Sci. Rep.* 7:2955. https://doi.org/10.1038/s41598-017-03116-x.

Conti F, Bartolucci F. 2016. The vascular flora of Gran Sasso and Monti della Laga National Park (Central Italy). *Phytotaxa* 256:1–119.

Conti F, Ciaschetti G, Martino LD, et al. 2019. An annotated checklist of the vascular flora of Majella National Park (Central Italy). *Phytotaxa* 412:1–90. https://doi.org/10.11646/phytotaxa.412.1.1

de la Mata R, Zas R, Bustingorri G, et al. 2022. Drivers of population differentiation in phenotypic plasticity in a temperate conifer: a 27-year study. *Evol. Appl.* 15:1945–1962. https://doi.org/10.1111/eva.13492

Farina L, Leonardi C. 2018. Note corologiche e tassonomiche su *Longitarsus springeri* Leonardi, 1975, con segnalazione di una forma cromatica inedita dei Monti Sibillini (Coleoptera, Chrysomelidae). *Bull. Soc. Entomol. Fr.* 123:185–192.

Folmer O, Black M, Hoeh W, et al. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* 3:294–299.

Gikonyo MW, Ahn S-J, Biondi M, et al. 2024. A radiation of *Psylliodes* flea beetles on Brassicaceae is associated with the evolution of specific detoxification enzymes. *Evolution* 78:127–145. https://doi.org/10.1093/evolut/qpad197

Giraudi C, Ehlers J, Gibbard P. 2004. The Apennine glaciations in Italy. *Quart. Glaciat. Chronol. Part Eur. Dev. Quat. Sci.* 2:215–224.

Giraudi C, Frezzotti M. 1997. Late Pleistocene glacial events in the central Apennines, Italy. *Quat. Res.* 48:280–290.

Guerrina M, Theodoridis S, Minuto L, et al. 2022. First evidence of post-glacial contraction of Alpine endemics: Insights from Berardia subacaulis in the European Alps. *J. Biogeogr.* 49:79–93. https://doi.org/10.1111/jbi.14282

Habel JC, Schmitt T, Müller P. 2005. The fourth paradigm pattern of post-glacial range expansion of European terrestrial species: the phylogeography of the Marbled White butterfly (Satyrinae, Lepidoptera). *J. Biogeogr.* 32:1489–1497.

Haubrich K, Schmitt T. 2007. Cryptic differentiation in alpine-endemic, high-altitude butterflies reveals down-slope glacial refugia. *Mol. Ecol.* 16:3643–3658.

Hewitt GM. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biol. J. Linn. Soc.* 58:247–276.

Hewitt GM. 1999. Post-glacial re-colonization of European biota. *Biol. J. Linn. Soc.* 68:87–112.

Hewitt GM. 2004. Genetic consequences of climatic oscillations in the Quaternary. *Philos. Trans. R Soc. Lond. B Biol. Sci.* 359:183–195; discussion 195.

Hoang DT, Chernomor O, Von Haeseler A, et al. 2018. UFBoot2: improving the ultrafast bootstrap approximation. *Mol. Biol. Evol.* 35:518–522.

Hoorn C, Perrigo A, Antonelli A. 2018. Mountains, climate and biodiversity. John Wiley & Sons.

Huemer P, Hebert PD. 2011. Cryptic diversity and phylogeography of high alpine Sattleria—a case study combining DNA barcodes and morphology (Lepidoptera: Gelechiidae). *Zootaxa* 2981:1–22.

Hughes C, Eastwood R. 2006. Island radiation on a continental scale: exceptional rates of plant diversification after uplift of the Andes. *Proc. Natl. Acad. Sci. U S A.* 103:10334–10339. https://doi.org/10.1073/pnas.0601928103.

Hughes CE, Atchison GW. 2015. The ubiquity of alpine plant radiations: from the Andes to the Hengduan Mountains. *New Phytol.* 207:275–282. https://doi.org/10.1111/nph.13230.

Hughes P, Woodward J, Gibbard P. 2006. Quaternary glacial history of the Mediterranean mountains. *Prog. Phys. Geogr.* 30:334–364.

Kahlke R-D, García N, Kostopoulos DS, et al. 2011. Western Palaearctic palaeoenvironmental conditions during the Early and early Middle Pleistocene inferred from large mammal communities, and implications for hominin dispersal in Europe. *Quat. Sci. Rev.* 30:1368–1395. https://doi.org/10.1016/j.quascirev.2010.07.020

Kalyaanamoorthy S, Minh BQ, Wong TK, et al. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat. Methods.* 14:587–589.

Kapli P, Lutteropp S, Zhang J, et al. 2017. Multi-rate Poisson tree processes for single-locus species delimitation under maximum likelihood and Markov chain Monte Carlo. *Bioinformatics* 33:1630–1638. https://doi.org/10.1093/bioinformatics/btx025

Katoh K, Misawa K, Kuma K, et al. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* 30:3059–3066.

Kenyeres Z, Rácz IA, Varga Z. 2009. Endemism hot spots, core areas and disjunctions in European Orthoptera. *Acta Zool. Cracoviensia-Ser. B Invertebr.* 52:189–211.

Leaché AD, Fujita MK. 2010. Bayesian species delimitation in West African forest geckos (*Hemidactylus fasciatus*). *Proc. Biol. Sci.* 277:3071–3077. https://doi.org/10.1098/rspb.2010.0662

Leigh JW, Bryant D. 2015. POPART: full-feature software for haplotype network construction. *Methods Ecol. Evol.* 6:1110–1116.

Leonardi C. 1975. Le *Psylliodes* appenniniche del museo civico di storia naturale di Verona (Coleoptera Chrysomelidae). *Boll. Mus. civ. stor. nat. Verona.* 2:51–90.

Leonardi C. 2007. Dati inediti sul genere *Psylliodes* Latreille, con descrizione di quattro nuove specie mediterranee (Coleoptera Chrysomelidae). *Atti Della Soc. Ital. Sci. Nat. E Mus. Civ. Storia Nat. Milano.* 148:161–240.

Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25:1451–1452.

Linder HP. 2008. Plant species radiations: where, when, why?*Philos. Trans. R Soc. Lond. B Biol. Sci.* 363:3097–3105. https://doi.org/10.1098/rstb.2008.0075.

Love SJ, Schweitzer JA, Woolbright SA, et al. 2023. Sky Islands are a global tool for predicting the ecological and evolutionary consequences of climate change. *Annu. Rev. Ecol. Evol. Syst.* 54:219–236. https://doi.org/10.1146/annurev-ecolsys-102221-050029

Magoga G, Fontaneto D, Montagna M. 2021. Factors affecting the efficiency of molecular species delimitation in a species-rich insect family. *Mol. Ecol. Resour.* 21:1475–1489. https://doi.org/10.1111/1755-0998.1335233565247

Magoga G, Sahin DC, Fontaneto D, et al. 2018. Barcoding of Chrysomelidae of Euro-Mediterranean area: efficiency and problematic species. *Sci. Rep.* 8:13398–13399.

Maiorano L, Falcucci A, Boitani L. 2006. Gap analysis of terrestrial vertebrates in Italy: priorities for conservation planning in a human dominated landscape. *Biol. Conserv.* 133:455–473.

Menchetti M, Talavera G, Cini A, et al. 2021. Two ways to be endemic. Alps and Apennines are different functional refugia during climatic cycles. *Mol. Ecol.* 30:1297–1310.

Minh BQ, Nguyen MAT, von Haeseler A. 2013. Ultrafast approximation for phylogenetic bootstrap. *Mol. Biol. Evol.* 30:1188–1195.

Montelongo T, Gómez-Zurita J. 2014. Multilocus molecular systematics and evolution in time and space of *Calligrapha* (Coleoptera: Chrysomelidae, Chrysomelinae). *Zool. Scr.* 43:605–628. https://doi.org/10.1111/zsc.12073

Nevado B, Contreras-Ortiz N, Hughes C, et al. 2018. Pleistocene glacial cycles drive isolation, gene flow and speciation in the high-elevation Andes. *New Phytol.* 219:779–793. https://doi.org/10.1111/nph.15243

Nguyen L-T, Schmidt HA, Von Haeseler A, et al. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol.* 32:268–274.

Ogilvie HA, Bouckaert RR, Drummond AJ. 2017. StarBEAST2 brings faster species tree inference and accurate estimates of substitution rates. *Mol. Biol. Evol.* 34:2101–2114.

Papadopoulou A, Anastasiou I, Vogler AP. 2010. Revisiting the insect mitochondrial molecular clock: the mid-Aegean trench calibration. *Mol. Biol. Evol.* 27:1659–1672.

Paradis E, Schliep K. 2019. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 35:526–528.

Platania L, Cardoso A, Anderson M, et al. 2024. New Caledonian rovers and the historical biogeography of a hyper-diverse endemic lineage of South Pacific leaf beetles. *Syst. Entomol.* 49:565–582. https://doi.org/10.1111/syen.12632

Price TD, Qvarnström A, Irwin DE. 2003. The role of phenotypic plasticity in driving genetic evolution. *Proc. Biol. Sci.* 270:1433–1440. https://doi.org/10.1098/rspb.2003.2372

Puillandre N, Brouillet S, Achaz G. 2021. ASAP: assemble species by automatic partitioning. *Mol. Ecol. Resour.* 21:609–620. https://doi.org/10.1111/1755-0998.13281

Rahbek C, Borregaard MK, Antonelli A, et al. 2019. Building mountain biodiversity: geological and evolutionary processes. *Science* 365:1114–1119.

Rambaut A, Drummond AJ. 2009. FigTree version 1.3. 1 [computer program]. Httptree Bio Ed Ac Uksoftwarefigtree. [accessed 2017 October 21].

Rambaut A, Drummond AJ, Xie D, et al. 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Syst. Biol.* 67:901–904.

Rannala B, Yang Z. 2003. Bayes estimation of species divergence times and ancestral population sizes using DNA sequences from multiple loci. *Genetics* 164:1645–1656.

Salvi D, Berrilli E, D'Alessandro P, et al. 2020. Sharpening the DNA barcoding tool through a posteriori taxonomic validation: the case of *Longitarsus* flea beetles (Coleoptera: Chrysomelidae). *Plos ONE* 15:e0233573. https://doi.org/10.1371/journal.pone.0233573

Salvi D, D'Alessandro P, Biondi M. 2019. Host plant associations in Western Palaearctic *Longitarsus* flea beetles (Chrysomelidae, Galerucinae, Alticini): a preliminary phylogenetic assessment. *Zookeys.* 856:101–114.

Salvi D, Harris DJ, Kaliontzopoulou A, et al. 2013. Persistence across Pleistocene ice ages in Mediterranean and extra-Mediterranean refugia: phylogeographic insights from the common wall lizard. *BMC Evol. Biol.* 13:1–18.

Sambrook J, Fritsch EF, Maniatis T. 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press.

Schmitt T. 2007. Molecular biogeography of Europe: Pleistocene cycles and postglacial trends. *Front. Zool.* 4:11–13.

Schmitt T. 2009. Biogeographical and evolutionary importance of the European high mountain systems. *Front. Zool.* 6:9–10.

Simon C, Frati F, Beckenbach A, et al. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann. Entomol. Soc. Am* 87:651–701.

Simpson GG. 1964. Species density of North American recent mammals. *Syst. Zool.* 13:57–73. https://doi.org/10.2307/2411825.

Smyčka J, Roquet C, Renaud J, et al. 2017. Disentangling drivers of plant endemism and diversification in the European Alps—a phylogenetic and spatially explicit approach. *Perspect. Plant Ecol. Evol. Syst.* 28:19–27.

Steinbauer MJ, Field R, Grytnes J-A, et al. 2016. Topography-driven isolation, speciation and a global increase of endemism with elevation. *Global Ecol. Biogeogr.* 25:1097–1107. https://doi.org/10.1111/geb.12469

Stephens M, Donnelly P. 2003. A comparison of bayesian methods for haplotype reconstruction from population genotype data. *Am. J. Hum. Genet.* 73:1162–1169.

Stephens M, Smith NJ, Donnelly P. 2001. A new statistical method for haplotype reconstruction from population data. *Am. J. Hum. Genet.* 68:978–989.

Storz JF, Scott GR, Cheviron ZA. 2010. Phenotypic plasticity and genetic adaptation to high-altitude hypoxia in vertebrates. *J. Exp. Biol.* 213:4125–4136. https://doi.org/10.1242/jeb.048181

Svenning J-C, Eiserhardt WL, Normand S, et al. 2015. The influence of paleoclimate on present-day patterns in biodiversity and ecosystems. *Annu. Rev. Ecol. Evol. Syst.* 46:551–572.

Taberlet P, Fumagalli L, Wust-Saucy A, et al. 1998. Comparative phylogeography and postglacial colonization routes in Europe. *Mol. Ecol.* 7:453–464.

Trifinopoulos J, Nguyen L-T, von Haeseler A, et al. 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Res.* 44:W232–W235.

Urbani F, D'Alessandro P, Frasca R, et al. 2015. Maximum entropy modeling of geographic distributions of the flea beetle species endemic in Italy (Coleoptera: Chrysomelidae: Galerucinae: Alticini). *Zool. Anz. J. Comp. Zool* 258:99–109. https://doi.org/10.1016/j.jcz.2015.08.002

Valle B, Di Musciano M, Gobbi M, et al. 2022. Biodiversity and ecology of plants and arthropods on the last preserved glacier of the Apennines mountain chain (Italy). *Holocene*. 32:853–865. https://doi.org/10.1177/09596836221096292

Yang Z, Rannala B. 2010. Bayesian species delimitation using multilocus sequence data. *Proc. Natl. Acad. Sci. U S A* 107:9264–9269. https://doi.org/10.1073/pnas.0913022107