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Conserving genetic diversity hotspots under climate change: Are protected areas helpful?

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ABSTRACT

The conservation of genetic diversity is a major target of the Kunming-Montreal Global Biodiversity Framework and of the EU Nature Restoration Law, as it provides populations with the potential to evolutionary adapt to the ongoing environmental challenges. However, genetic diversity has often been neglected in the conservation planning, and data on the extent to which it is currently preserved by protected areas are scanty. Here, we assessed the efficacy of protected areas in preserving genetic diversity hotspots under global change. Focusing on the Italian peninsula inside the Mediterranean global biodiversity hotspot, we (i) investigated the patterns of genetic diversity of endemic terrestrial vertebrates, (ii) assessed how much genetic diversity is currently covered by protected areas considering both nuclear and mitochondrial DNA, and (iii) estimated the impact of projected range shifts caused by climate changes on the conservation of genetic diversity patterns. We found that protected areas cover <20 % of the areas of high genetic diversity for most of the investigated taxa, and fail to cover almost all multispecies genetic diversity hotspots. Furthermore, our results showed that mitochondrial DNA is not a reliable proxy for nuclear genome diversity, and its use in spatial conservation planning might lead to ineffective initiatives. Finally, we estimated that the extent of genetic diversity covered by protected areas will dramatically decline in the near future (2050). These results identify major gaps in current protection of genetic diversity and provide concrete guidelines to plan area-based conservation initiatives that meet biodiversity conservation targets for 2030.

1. Introduction

Human driven global changes are threatening biological diversity at all its levels of organization (Cardoso et al., 2020; Sage, 2020; CBD, 2022). Above the species level, global changes are altering biological communities and corrupting the natural processes regulating ecosystem functions (Castro et al., 2015; Rico-Sánchez et al., 2020). At the species level, they are causing widespread extinctions at unprecedented rates in all groups of living organisms for which data are available (Butchart et al., 2010; Román-Palacios and Wiens, 2020). Below the species level, they are eroding genetic diversity within populations (Leigh et al., 2019), compromising their capacity to adapt to changing environments (Kardos et al., 2021). Despite the importance of protecting all the levels of biodiversity being undisputed (strategic goal C, Convention on Biologic Diversity CBD, 2022), most conservation actions proposed to date focused on the species level and did not get the same achievements at the other levels.

Protected areas provide the best option to protect all the levels of biodiversity at once (Maxwell et al., 2020). By applying specific regulations to limit human activities in certain areas, protected areas are expected to counteract habitat degradation (Geldmann et al., 2019; Joppa and Pfaff, 2010; but see Maiorano et al., 2008), to conserve ecosystem functions (Castro et al., 2015), and to maintain viable population size for endangered species (Watson et al., 2014; Maxwell et al., 2020). However, so far protected areas have been mainly designed to protect specific endangered taxa or species assemblages (*e.g.,* the Gir Forest Wildlife Sanctuary in India, established to save the last Asian lions), or to limit the alteration of iconic landscapes (*e.g.*, the Yellowstone National Park), *de facto* circumscribing their positive effects on a very limited portion of biodiversity. Despite the indisputable

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conservation value of these areas, a strong taxonomic and geographical bias has been outlined (Venter et al., 2018a, 2018b). Gap analyses and systematic conservation planning have been developed to overcome this bias and to achieve more balanced and effective benefits of the protected areas on biodiversity (Margules and Pressey, 2000; Rodrigues et al., 2004; Rodríguez et al., 2007; Maiorano et al., 2006, 2015). However, few applications have been focused on biodiversity above the species level (*i.e.* community level; O'Connor et al., 2021), and even less evaluated how protected areas perform in protecting the genetic level of biodiversity (but see Schmidt et al., 2024). The lack of information on the coverage of genetic diversity by protected areas prevents to evaluate their long-term effectiveness under a global change scenario (Pearman et al., 2024).

Genetic diversity, indeed, provides populations with the evolutionary potential to face environmental challenges (Frankham, 2010; DeWoody et al., 2021; Kardos et al., 2021; Andrello et al., 2022). Populations with higher levels of genetic diversity have proved to be the less vulnerable to the detrimental consequences of genetic drift (van Oosterhout, 2020) and, at the same time, the more likely to evolve traits increasing population-level fitness in response to novel environmental conditions (Allendorf et al., 2012; Forester et al., 2022). For this reason, the most recent international conservation policies, such as the recently adopted Kunming-Montreal Global Biodiversity Framework (KMGBF Target 4; CBD, 2022) and the European Nature Restoration Law (European Commission, 2022), committed to conserve genetic diversity for all species. Therefore, mapping the spatial patterns of genetic diversity, and identifying hotspots of genetic diversity for multiple taxa (Hewitt, 2004, 2011; Hampe and Petit, 2005) would provide valuable information to identify priority areas promoting the long-term conservation of biodiversity in an evolutionary perspective (*i.e.,* adding a temporal and dynamic dimension to the area-based conservation approach). The last two decades have seen a substantial increase in the availability of genetic data, and a few attempts to map the spatial patterns of genetic diversity have been made (*e.g.*, Miraldo et al., 2016; Blanchet et al., 2017; Carvalho et al., 2017; Kling et al., 2019; Mazel et al., 2018; Dapporto et al., 2022; French et al., 2023; Schmidt et al., 2024). Yet, these studies identified patterns of genetic diversity over global or regional scales, relying on genetic information mainly coming from mitochondrial genes, which is by far the most available genetic marker (Schmidt and Garroway, 2021). Nonetheless, the integration of these data in biodiversity conservation planning shows some limitations. First, these studies identified hotspots of genetic diversity over large areas, a spatial resolution than can hardly be implemented in practical conservation management (Cañadas and Vázquez, 2014). Second, patterns of variation at mitochondrial genes may not reflect the pattern of diversity at the genome level (Ballard and Whitlock, 2004; Zink and Barrowclough, 2008; Edwards and Bensch, 2009; Schmidt and Garroway, 2021). Last, but not least, most of these studies computed genetic diversity metrics (usually phylogenetic diversity or nucleotide diversity) across all the species within a given cell, resulting in an estimate of the mean genetic divergence of species in a place rather than a realistic assessment of genetic diversity of population units (Carvalho et al., 2017). Therefore, despite the acknowledged value and the increased availability of genetic data (Leigh et al., 2024), a solid framework to incorporate them in biodiversity conservation is still missing (Nielsen et al., 2023; Paz-Vinas et al., 2023).

With this study, we aim to assess the effectiveness of protected areas in preserving the hotspots of genetic diversity under global change. We focused on the Italian peninsula because it is a well-known hotspot of biodiversity where most of species studied have shown complex and scattered patterns of intraspecific genetic structure (Canestrelli et al., 2014; Hewitt, 2011; Schmitt et al., 2021). To this aim, we collected all the available genetic information for terrestrial vertebrates endemic to the Italian peninsula and mapped their spatial pattern of mitochondrial and nuclear genetic diversity. Then, after identifying the location of the main genetic diversity hotspots, we assessed: i) the coverage of genetic diversity hotspots by the Italian protected area network, ii) the concordance between information gathered from mitochondrial and nuclear genetic data, and iii) the effectiveness of the current protected area network in protecting genetic diversity in the future, under alternative climate change scenarios. This study is addressed to provide relevant information to implement the conservation of genetic diversity, according to the Kunming-Montreal Global Biodiversity Framework and the EU Nature Restoration Law.

2. Methods

2.1. Study area and taxa definition

Our study area covers the entire Italian Peninsula, islands excluded, going from the southern Alps to the southernmost Italian shores (roughly 131,000 km^2, Supplementary Fig. S1). This region has high topographic heterogeneity, being characterized by an extended coastline and by the presence of the Apennine Mountain chain (running through 1200 km along the whole length of the peninsula), and encompasses several bioclimatic regions (Mediterranean, Oceanic, Continental, Tundra; Peel et al., 2007). We focused our analyses on terrestrial non-volant vertebrates endemic to the Italian peninsula (islands excluded). To account for intraspecific diversity and local adaptations in the niche evolution (Zimmermann et al., 2010), we used as unit of analysis single independent evolutionary lineages rather than species. We selected 22 distinct evolutionary lineages genetically isolated from co-specific populations, and entirely distributed within the Italian peninsula. We excluded the cold-adapted taxa (*i.e. Ichthyosaura alpestris* and *Rana temporaria*) because we expected contrasting signals compared to the other temperate species. The final dataset included 16 lineages of amphibians, three reptiles, three small mammals (see Supplementary Table S1 for the literature used to identify each lineage's genetic and geographic boundaries). We used the taxonomic name available in the International Union for Conservation of Nature (IUCN) Red List (https://www.iucnredlist.org); for intraspecific lineages not identified at the subspecific level, we used the taxonomic names coupled with a geographical epithet identifying the distribution of the lineage in the Italian peninsula (*e.g.*, northern *vs.* southern lineage).

2.2. Genetic diversity data

To map the fine-scale distribution of genetic diversity across the Italian peninsula, we collected all the genetic data with accurate geographic annotation available from previous studies on the investigated taxa (Bisconti et al., 2024; Canestrelli et al., 2006a,b, 2007, 2008, 2012a,b, 2014; Canestrelli and Nascetti, 2008; Chiocchio et al., 2019, 2021, 2022; Maura et al., 2014; Salvi et al., 2013; Senczuk et al., 2017; Steinfartz et al., 2000; see Supplementary Table S1). For each lineage, we retrieved data from both nuclear and mitochondrial genetic markers. As nuclear markers, we included data from microsatellites, allozymes, and single nucleotide polymorphisms (SNPs), which are commonly used to produce the most used estimators of population genetic diversity: allelic richness, gene diversity, and expected heterozygosity. As mitochondrial markers, we included data from sequence markers, which are commonly used to estimate haplotype and nucleotide diversity.

Among the many estimators of population genetic diversity, we choose to focus on allelic richness for nuclear makers and nucleotide diversity for mitochondrial markers. Allelic richness is a measure of the number of alleles for locus, averaged across loci after accounting for the locus with the lower number of alleles (Kalinowski, 2004). Allelic richness has been selected among the others because it is more sensitive to the presence of rare genetic variants and in detecting population bottlenecks, and therefore, this estimate found wider use in conservation genetic applications (Cornuet and Luikart, 1996; Petit et al., 1998). Nucleotide diversity (π) is the average number of nucleotide differences per site between two randomly chosen sequences from the same

population (Tajima, 1993). Nucleotide diversity has been chosen because it is less sensitive to the variation of sequence length among the different studies, and because it was widely used to map mitochondrial genetic diversity (Miraldo et al., 2016). If available, the estimators were directly imported from the study; if not, allelic richness was estimated using the diveRsity R package (Keenan et al., 2013), while nucleotide diversity was estimated using DNASP 5.10 (Librado and Rozas, 2009). Data from location containing less than three individuals were excluded from the analyses.

2.3. Species distribution models

To obtain continuous maps of current and future distribution for each lineage, we used presence-only correlative species distribution models (SDMs) in an ensemble forecasting approach implemented through package biomod2 (Thuiller et al., 2014) in R (R Core Team, 2023).

2.3.1. Calibration areas

To calibrate our SDMs, we defined lineage-specific calibration areas considering the geographical area available to each lineage for post-glacial re-colonization (Machado-Stredel et al., 2021). Most lineages ($n = 15$) spanned the entire Italian peninsula and therefore the calibration area included all continental Italy (Supplementary Fig. 2a, Supplementary Table 1). For those lineages whose current distribution included Sicily ($n = 3$) we also included the island in the calibration area (Supplementary Fig. S2b, Supplementary Table 1). For *Bufo bufo* southern lineage, which has never naturally occurred into the Po plain (Chiocchio et al., 2021), we defined a calibration area going from the extreme southern regions up to the northern Apennine (defined following Falcucci et al., 2007;Supplementary Fig. S2c, Supplementary Table S1). For the lineages whose current distribution was limited to the Calabrian peninsula (n = 3) we restricted the calibration area to southern Italy using the Sangro and Volturno rivers as northern boundaries (Supplementary Fig. S2d in, Supplementary Table S1).

2.3.2. Occurrence and background data

For each lineage, we obtained occurrence data from different sources: the datasets associated with the phylogeographic studies (Supplementary Table S1); the CKmap 5.3.8 database, reporting historical occurrences for Italy validated by taxon-specific experts (Stoch, 2000-2005); the citizen sciences database Global Biodiversity Information Facility (www.GBIF.org) and Observado (www.observation.org). Citizen science databases were filtered by removing occurrences not associated with accurate photo, date, and coordinates, and keeping only "research grade" data (*i.e.* species occurrences confirmed by at least 3 different users). From all databases, we excluded all occurrences with a spatial accuracy $\leq$1 km and collated all data into a single occurrence dataset for each lineage.

To limit the impact of data clustering on the species distribution models (Geldmann et al., 2019) we used a spatial thinning approach (Aiello-Lammens et al., 2015). We excluded from the calibration dataset all occurrences clustered together based on a nearest-neighbor distance (NND) calculated as follows: for each lineage, we generated 10,000 sets of random points covering the model calibration area; for each set of random points, we calculated the average NND obtaining 10,000 values for each lineage. The average NND over the 10,000 replicas was used as thinning distance in the r package spThin (Aiello-Lammens et al., 2015), to obtain 5 alternative occurrence datasets for each lineage. These 5 datasets were used in alternative model calibrations in our ensemble forecast. For each calibration dataset we also obtained 5 alternative evaluation datasets including all occurrences excluded by the spatial thinning procedure.

To calibrate the SDMs, we coupled each of the 5 occurrence datasets with a set of background points for each lineage. To account for the uneven distribution of the sampling efforts associated with our opportunistic presence data, we followed a target group approach (Ranc et al., 2017) and used as background the occurrences of amphibians, reptiles, and mammals within the calibration area from Maiorano et al. (2013). We pooled together the background of amphibians and reptiles, assuming that they share the same observer bias. For the three species with a distribution limited to southern Italy the background points were too scanty to calibrate a reliable SDM, and therefore we calibrated these models with 4000 random background points (Supplementary Table S1).

2.3.3. Bioclimatic predictors

To represent the current climate (average for years 1970–2000), we obtained 19 bioclimatic variables from the WorldClim 2.1 Database (1 km^2 resolution; Fick and Hijmans, 2017). To select one set of bioclimatic variables for each lineage, we calculated their collinearity on the variables extracted from the presence and background points and retained only those whose VIF (Variance Inflation Factor) was <5 to avoid multicollinearity (Supplementary Table S2, Zuur et al., 2010).

For future climate scenarios, we considered the four time frames available on WorldClim 2.1 Database: 2021-2040, 2041-2060, 2061-2080, 2081-2100. For each time frame, we extracted the same bioclimatic variables with the same spatial resolution (1 km^2), considering five General Circulation Models (GCMs) available under the IPCC6 (the sixth Assessment Report of the Intergovernmental Panel on Climate Change; IPCC, 2022): IPSL-CM6A-LR, MIROC6, MPI-ESM1-2-HR,MRI-ESM2-0,UKESM 1-0-LL, following the guidelines of Sanderson et al. (2015). For each GCM, we considered three Representative Concentration Pattern (RCP) – Shared Socio-economic Pathways (SSP) scenarios resulting in increasing global temperature rise by 2100 (IPCC, 2022): SSP1-2.6 (below 2 °C), SSP2-4.5 (between 2 °C and 3 °C), SSP5-8.5 (between 2.6 °C and 4.8 °C).

2.3.4. Ensemble modelling

We calibrated a SDM for each lineage following an ensemble modelling approach (Araújo et al., 2019)with five alternative occurrence datasets (obtained through the thinning) and five algorithms: generalized linear models (GLM), generalized additive models (GAM), generalized boosted models (GBM), multivariate adaptive regression splines (MARS), and maximum entropy (MAXENT).

We evaluated model performance using both an internal and an external model evaluation procedure. For each of the 5 sets of occurrences each model run was evaluated by calculating the AUC within a 5-fold cross-validation with 80 % of the data used for calibration and 20 % for internal evaluation. Only the runs with AUC > 0.7 were used to obtain a single model calculated as the weighted average across all cross-validation runs (Swets, 1988; Thuiller et al., 2014). Each of the five weighted average models was evaluated through AUC (external evaluation) considering the five alternative evaluation datasets obtained through the thinning.

For each set of occurrences, we obtained continuous suitability maps by projecting the final model to current climate conditions. We binarized each projection using the maximum true skill statistic (TSS, Liu et al., 2016) threshold. We pooled the five binary models into a single presence/absence map projected over each lineage range obtained from IUCN (https://www.iucnredlist.org/resources/spatial-data-download) by classifying as presence all pixels where at least one projection predicted a presence.

We projected the final model to future climate conditions considering three future socio-economic scenarios and the four-time frames for each of the 5 GCMs. We binarized each projection using the maximum TSS (Liu et al., 2016) threshold. We pooled the five binary models into a single presence/absence map by classifying as presence all pixels where at least one projection predicted a presence. Lastly, we obtained a binary presence/absence map considering as presence all pixels where at least three GCMs predicted a presence. Assuming no dispersal possibility across the highly fragmented Italian landscape, we cropped the resulting map of future distribution by the IUCN range of each lineage.

2.4. Spatial interpolation of genetic diversity and identification of area hotspots

For each lineage, we interpolated allelic richness and nucleotide diversity data using an inverse distance approach (ArcGIS Pro 3.2; ESRI ©) over all pixels classified as presence in our SDMs projected under current climate. We evaluated the reliability of the interpolations using a jackknife approach, excluding one of the points from the analyses, performing the interpolation on all the others, and measuring the root mean square error among the estimate and the real measure.

We defined as areas of high genetic diversity for each lineage (*i.e.* single-lineage genetic diversity hotspot) those holding the top 2.5 % of diversity values. We estimated the future distribution of the areas of high genetic diversity by cropping the interpolations of genetic data into the future range obtained by SDMs projections. To identify the hotspots of genetic diversity for multiple lineages, we summed the areas of high genetic diversity for each lineage and considered as hotpots those containing the top 50 % of the obtained values.

2.5. Concordance among mitochondrial and nuclear genetic data

We assessed if the fine-scale spatial patterns of genetic diversity coming from mitochondrial genetic data mirror the spatial patterns of genetic diversity coming from the nuclear genetic data, and thus can be used to inform spatial conservation planning. To do this, we compared the location of single-lineage genetic diversity hotspots obtained from either mitochondrial or nuclear data and calculated the overlap (km^2) between the two hotspot areas for each lineage.

2.6. Gap analysis

We applied a gap analysis to evaluate the current coverage of lineage distribution and genetic diversity by the current system of protected areas, that includes National and Regional Parks and Reserves (*https://www.mase.gov.it/pagina/elenco-ufficiale-delle-aree-naturali*). We also estimated the future coverage according to the different emission scenarios. In particular, for each time frame we calculated: 1) the percentage of suitable range covered by protected areas (*i.e.* coverage); 2) the percentage of the areas of high genetic diversity covered by protected areas; 3) the percentage of the genetic diversity hotspot covered by protected areas. The analyses were performed with packages "raster" (Hijmans et al., 2005), "SpatialEco" (Evans et al., 2021), and "rgdal" (Bivand, 2022) in R (R Core Team 2022).

3. Results

3.1. SDMs evaluation

All ensemble models had a good predictive performance (mean TSS = 0.580, sd = 0.176, and mean AUC = 0.890, sd = 0.068). Lineage-specific AUC and TSS values are listed in Supplementary Table S3 and S4. For all the analyzed taxa, our SDMs provide a reliable picture of the current species distribution, matching well with the most updated map of species distribution (Lanza, 2007; Sindaco et al., 2006). Distribution maps for each lineage under current and future conditions are available in Zenodo (to be populated upon acceptance).

3.2. Spatial interpolation of genetic diversity and concordance among mitochondrial and nuclear data

The spatial interpolations of genetic diversity values had a mean RMSE of 0.07 (sd = 0.04) for nuclear diversity and 0.27 (sd = 0.11) for mitochondrial diversity. Lineage-specific RMSE values are listed in Supplementary Table S5. The maps of intraspecific genetic diversity for each lineage are available in Zenodo (to be populated upon acceptance).

Lineage-specific areas of high genetic diversity according to nuclear and mitochondrial data had a mean surface of 4047 (sd = 3058) and 5235 km^2 (sd = 4991), respectively, and they overlapped for an average of 9 % of their cumulative surface (sd = 11 %, Table 1). Predicted multi-lineage hotspots of genetic diversity had a surface of 1057 km^2 when

Table 1
Current coverage of suitable habitat and of genetic diversity hotspots: surface (km^2) within each lineage and percentage of it that falls into protected areas network (within parentheses, PAs) for current time (1970–2000). Mitochondrial diversity: nucleotide diversity; nuclear diversity: allelic richness.

Lineage	Suitable range in km^2 (PA coverage%)	Mitochondrial diversity in km^2 (PA coverage%)	Nuclear diversity in km^2 (PA coverage%)
Amphibians			
Bombina pachypus	108,856 (10 %)	3638 (12 %)	3767 (6 %)
Bufo bufo C	85,160 (15 %)	11,790 (10 %)	3609 (11 %)
Bufo Bufo S	130,965 (14 %)	4830 (17 %)	7075 (18 %)
Hyla intermedia N	108,290 (7 %)	9478 (2 %)	601 (7 %)
Hyla intermedia S	150,724 (11 %)	7550 (10 %)	1296 (0 %)
Lissotriton italicus N	64,083 (17 %)	2630 (9 %)	854 (42 %)
Lissotriton italicus S	12,940 (8 %)	362 (0 %)	877 (0 %)
Lissotriton vulgaris meridionalis	182,306 (8 %)	7032 (24 %)	na
Rana dalmatina	45,636 (26 %)	1051 (5 %)	1747 (39 %)
Rana italica	139,492 (13 %)	3351 (33 %)	18,879 (28 %)
Rana lessonae	171,694 (9 %)	3556 (6 %)	na
Salamandra salamandra	82,438 (22 %)	3066 (33 %)	14,947 (9 %)
Salamandrina perspicillata	81,153 (10 %)	1421 (8 %)	3666(9 %)
Salamandrina terdigitata	22,066 (32 %)	1793(29 %)	1613 (21 %)
Triturus carnifex N	137,680 (9 %)	2783 (1 %)	2438 (0 %)
Triturus carnifex S	130,753 (11 %)	4641 (4 %)	11,603 (8 %)
Reptiles			
Podarcis muralis	32,494 (32 %)	2537 (43 %)	na
Podarcis siculus	168,336 (9 %)	8655 (5 %)	4283 (4 %)
Testudo hermanni	107,675 (8 %)	4078 (5 %)	1527 (15 %)
Mammals			
Myodes glareoulus	12,349 (34 %)	3273 (43 %)	8950 (33 %)
Sciurus meridionalis	11,715 (40 %)	2041 (58 %)	4437 (53 %)
Talpa romana	112,622 (13 %)	982 (8 %)	7300 (12 %)

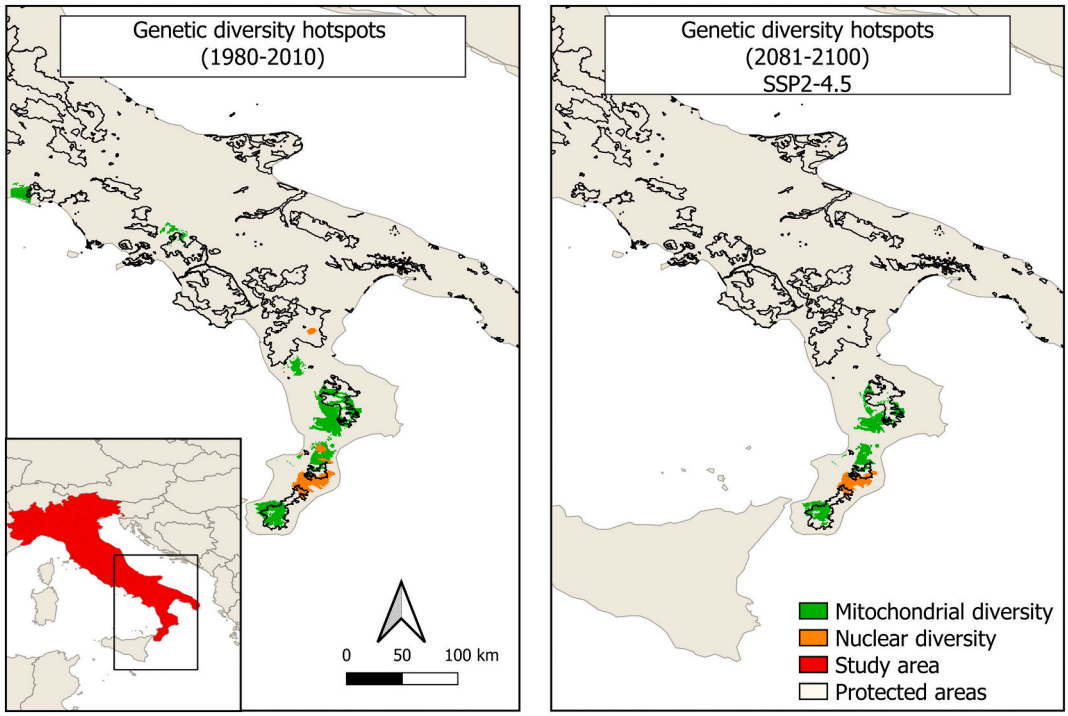

Fig. 1. Hotspots of genetic diversity for 22 lineages of terrestrial non-volant vertebrates endemic to the Italian peninsula under current conditions (left panel), and projection of the hotspots in the future (2081–2100) under a climate change scenario following the Socio-economic Pathways SSP5–8.5 (right panel).

considering nuclear data, and of 4115 km^2 when considering mitochondrial data (Fig. 1). They overlapped for 3 % of their cumulative surface (165 km^2).

3.3. Gap analysis

3.3.1. Suitable range

Currently, protected areas cover on average 16 % (sd = 10 %, minimum = 7 % - *Hyla intermedia* northern lineage - maximum = 40 % - *Sciurus meridionalis*) of the estimated suitable range for each lineage. However, for 73 % (n = 16) of the considered lineages, protected area coverage was <20 % (Fig. 2, Table 2).

According to the scenario SSP 2–4.5 by 2100, 5 % (n = 1; *Hyla intermedia* northern lineage) of the lineages will gain suitable range (+23 %) and protected surface (+51 % Fig. 2, Table S6 in Supplementary). Meanwhile, 95 % (n = 21) of the lineages will lose suitable range (mean = −52 %; sd = 31 %; Fig. 2, Supplementary Table S6). Of these, 19 % (n = 4) will gain protected surface (mean = +15 %, sd = 9 %) and 81 % (n = 18) will lose protected surface (mean = −47 %, sd = 27 %, Fig. 2, Table S6 in Supplementary).

According to the scenario SSP 1–2.6, by 2100 41 % of the lineages (n = 9) will gain suitable range (mean = +11 %, sd = 9 %) and protected surface (mean = +28 %, sd = 20 %; Fig. S3 in Supplementary). Meanwhile, 59 % of the lineages (n = 13) will lose suitable range (mean = −24 %, sd = 14 %, Fig. S3 and Table S6 in Supplementary). Of these lineages, 31 % (n = 4) will gain protected surface (+6 %, sd = 7) and 69 % will lose protected surface (mean = −19 %, sd = 10 %; Fig. S3 and Table S6 in Supplementary).

According to the scenario SSP 5–8.5 by 2100, 5 % (n = 1; *Hyla intermedia* northern lineage) of the lineages will gain suitable range (+31 %) and protected surface (+78 % Fig. S4, Table S6 in Supplementary). Meanwhile, 95 % (n = 21) of the lineages will lose suitable range (mean = −72 %, sd = 27 %, Fig. S4, Table S6 in Supplementary). Of these lineages, 5 % (n = 1; *Triturus carnifex* northern lineage) will gain protected surface (+4 %) and 95 % (n = 20) will lose protected surface (mean = −63 %, sd = 31 %; Supplementary Fig. S4, Supplementary Table S6).

3.3.2. Nuclear genetic diversity

The current coverage protected areas coverage of the lineage-specific areas of high nuclear diversity was on average 17 % (sd = 16 %, minimum = 0 % - *Lissotriton italicus* southern lineage - maximum = 53 % - *Sciurus meridionalis*) and was <20 % for 68 % of the lineages (n = 13, Fig. 2, Table 2).

According to the scenario SSP 2–4.5, by 2100, 11 % (n = 2) of the areas of high nuclear diversity will maintain their total and protected surface, meanwhile, 89 % (n = 17) of them will lose surface (mean = −62 %, sd = 31, Fig. 2, Table S7 in Supplementary). Of these, 6 % (n = 1, *Podarcis siculus*) will maintain its protected surface and 94 % (n = 16) will lose protected surface (mean = −58 %, sd = 40 %; Fig. 2, Table S7 in Supplementary). Among the 19 lineages included in this analysis, 16 % (n = 3) will completely lose their areas of high nuclear diversity by 2100 according to scenario SSP 2–4.5 (Fig. 2, Table S7 in Supplementary).

According to the scenario SSP 1–2.6, by 2100 21 % (n = 4) of the areas of high nuclear diversity will maintain their total and protected surface (Fig. S2 and Table S7 in Supplementary). Meanwhile, 79 % (n = 15) of the areas of high nuclear diversity will lose their total surface (mean = −28 %, sd = 19, Fig. S3 and Table S7 in Supplementary). Of these, 7 % (n = 1, *Lissotriton italicus* northern lineage) will maintain its protected surface and 93 % (n = 14) will lose protected surface (mean = −31 %, sd = 34 %; Fig. S2 and Table S7 in Supplementary).

According to the scenario SSP 5–8.5, by 2100, 11 % (n = 2) areas of high nuclear diversity will maintain their total and protected surface. Meanwhile 89 % (n = 17) of the areas of high nuclear diversity will lose total (mean = −83 %, sd = 21, Fig. S3 and Table S7 in Supplementary) and protected (mean = −69 %, sd = 37 %; Fig. S4 and Table S7 in Supplementary) surface. Among the 19 lineages included in this analysis, 37 % (n = 7) will completely lose their areas of high nuclear diversity by 2100 according to scenario SSP 5–8.5 (Fig. S2 and Table S7 in Supplementary).

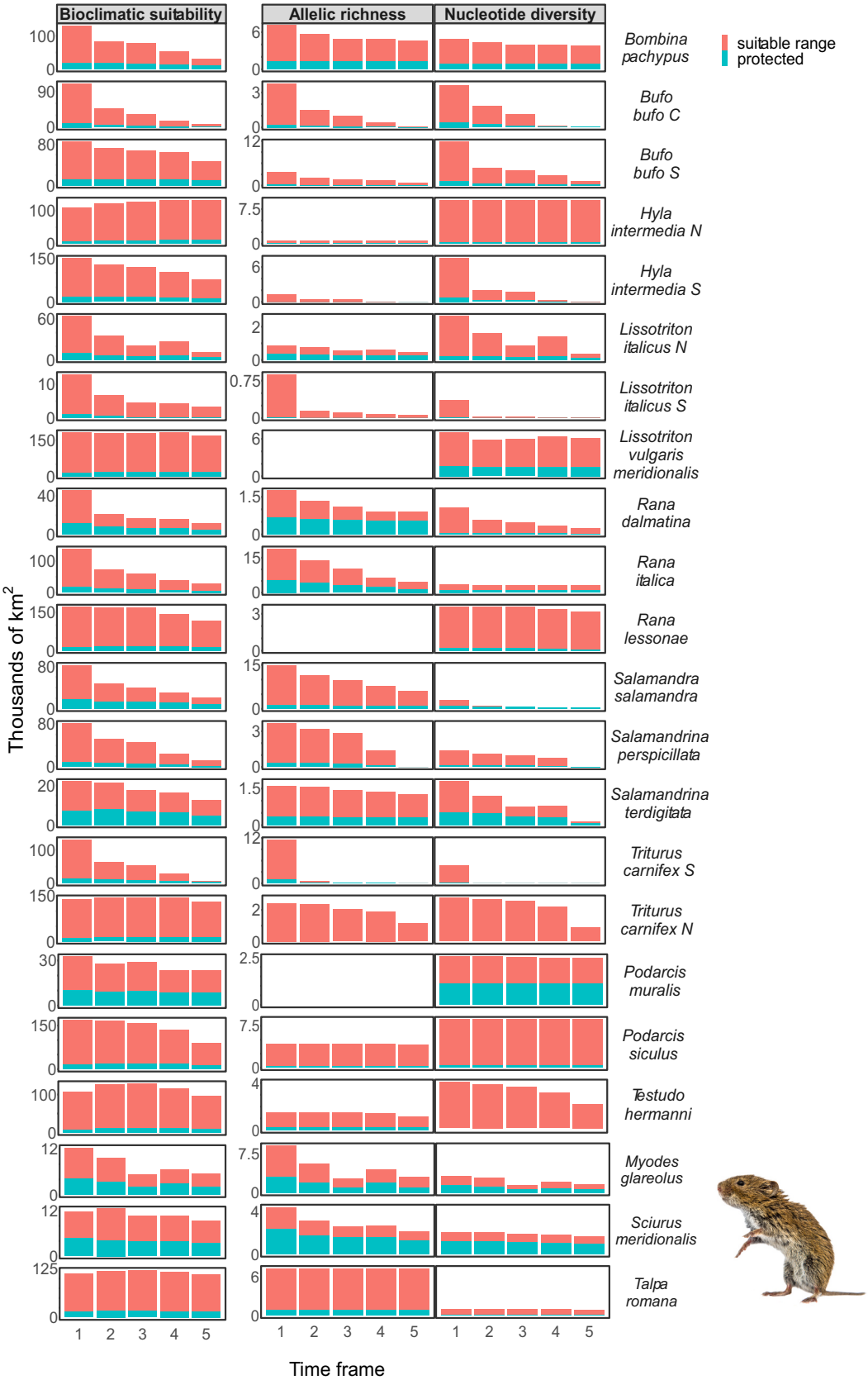

Fig. 2. Reduction of suitable range and areas of high genetic diversity through time for the 16 amphibian, 3 reptile and 3 mammal taxa considered in this study: suitable range (first column), area of high nuclear genetic diversity (second column) and area of high mitochondrial genetic diversity (third column) in the current (1970–2000) and future time frames (21–40, 41–60, 61–80, 81–100) under Socio-economic Pathways scenario SSP2–4.5. C = central Italy taxon, S = southern Italy taxon, N = northern Italy taxon. The extent of the area within the IUCN species range is represented in red; the coverage by protected areas is represented in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Current surface (km^2) of lineage-specific nuclear and mitochondrial genetic diversity hotspots, and surface of spatial overlap between them.

Lineage	Areas of high mitochondrial diversity (km^2)	Areas of high nuclear diversity (km^2)	Overlap (km^2)	Percent overlap
Amphibians				
Bombina pachypus	4830	7075	4075	34
Bufo bufo C	3638	3767	0	0
Bufo Bufo S	11,790	3609	2442	16
Hyla intermedia N	9478	601	194	2
Hyla intermedia S	7550	1296	128	1
Lissotriton italicus N	2630	854	150	4
Lissotriton italicus S	362	877	306	25
Lissotriton vulgaris meridionalis	7032	–	–	–
Rana dalmatina	1051	1747	4	0
Rana italica	3351	18,879	714	3
Rana lessonae	3556	–	–	–
Salamandra salamandra	3066	14,947	0	0
Salamandrina perspicillata	1421	3666	1159	23
Salamandrina terdigitata	1793	1613	0	0
Triturus carnifex N	2783	2438	0	0
Triturus carnifex S	4641	11,603	4641	29
Reptiles				
Podarcis muralis	2537	–	–	–
Podarcis siculus	8655	4283	409	3
Testudo hermanni	4078	1527	625	11
Mammals				
Myodes glareoulus	3273	8950	2094	17
Sciurus meridionalis	2041	4437	0	0
Talpa romana	982	7300	59	1
Mean	**4175**	**5235**	**895**	**9**
SD	**2948**	**4991**	**1374**	**11**

The total area of current nuclear diversity hotspots was 1057 km^2, with a PA coverage of 30 % (321 km^2). In 2100 such area is predicted to drop by 25 % (792 km^2) with a PA coverage of 30 % (236 km^2) according to SSP 2–4.5 (Supplementary Table S9). Results about multi-lineage PA coverage trends for SSP 1–2.6 and SSP 5–8.5 are shown in Supplementary Table S9.

3.3.3. Mitochondrial genetic diversity

Current protected area coverage of areas of high mitochondrial diversity was on average 17 % (sd = 16 %, minimum 0 % - *Lissotriton italicus* southern lineage, maximum = 58 % - *Sciurus meridionalis*), but for 68 % (n = 15) of the considered lineages protected area coverage was <20 % (Table 2, Fig. 2).

According to the scenario SSP 2–4.5, by 2100, 9 % (n = 2) of the lineage-specific areas of high mitochondrial diversity will maintain their total and protected surface, meanwhile and 91 % (n = 20) will lose surface (mean = −58 %, sd = 39 %, Fig. 2 Table S8 in Supplementary). Of these lineages, 15 % (n = 3) will maintain their protected surface and 90 % (n = 17) will lose protected surface (mean = −63 %, sd = 34 %; Fig. 2 Table S8 in Supplementary).

Among the 22 lineages included in this analysis, 14 % (n = 3) will completely lose their areas of high mitochondrial diversity by 2100 according to scenario SSP 2–4.5 (Fig. 2 Table S8 in Supplementary).

According to the scenario SSP 1–2.6, by 2100, 27 % (n = 6) of the lineage-specific areas of high mitochondrial diversity will maintain their total and protected surface, meanwhile, 73 % (n = 16) of them will lose total surface (mean = −29 %, sd = 26; Fig. S3 and Table S8 in Supplementary). Of these, 25 % (n = 4) will maintain their protected surface and 75 % (n = 12) will lose protected surface (mean = −16 %, sd = 22 %; Fig. S2 and Table S8 in Supplementary).

According to the scenario SSP 5–8.5, by 2100, 5 % (n = 1, *Hyla intermedia* northern lineage) of the areas of high mitochondrial diversity will maintain its total and protected surface, and 95 % (n = 21) of them will lose surface (mean = −70 %, sd = 40 %). Of these, 9 % (n = 2) will maintain their protected surface and 91 % (n = 19) will lose protected surface (mean = −77 %, sd = 35 %; Fig. S4 and Table S8 in Supplementary). Among the 22 lineages included in this analysis, 32 % (n = 7) will completely lose their areas of high mitochondrial diversity by 2100 according to scenario SSP 5–8.5 (Fig. S2 and Table S8 in Supplementary).

The total area of current mitochondrial diversity hotspots was 4115 km^2, with a PA coverage of 34 % (1393 km^2). In 2100 such area is predicted to drop by 48 % (2145 km^2) with a PA coverage of 38 % (814 km^2) according to SSP 2–4.5 (Supplementary Table S9). Results about multi-lineage PA coverage trends for SSP 1–2.6 and SSP 5–8.5 are shown in Supplementary Table S9.

4. Discussion

Despite the acknowledged value of genetic diversity in conservation biology (Frankham, 2010; Andrello et al., 2022; Nielsen et al., 2023; Pearman et al., 2024) and the increased availability of genetic data, recently overviewed by Schmidt et al. (2024), an assessment of its protection status is still lacking. In this study, we evaluated the level of protection of genetic diversity within a biodiversity-rich region, the Italian peninsula, by mapping the spatial pattern of genetic diversity for endemic terrestrial non-volant vertebrates and contrasting them to the current network of protected areas. Clearly our results heavily depend on the modelling approach we used, and upon the data available. In particular, the data available on species presence have been collected from different sources and with different sampling schemes; clustering and spatial biases may therefore represent a problem. Using a data thinning approach coupled with a target group approach for background locations should have limited these problems with model calibration. To calculate genetic diversity, we collected as much data as possible, but any spatial interpolation will invariably depend on the spatial location of the records being used. Thus, to avoid extrapolating genetic diversity data over large unsampled areas, we chose an extremely conservative threshold (top 2.5 %) in the definition of our hotspots of diversity.

Our results showed that protected areas cover only a very small amount of the areas holding the most intraspecific genetic diversity. By overlapping these areas for all the investigated lineages, we were able to identify the hotspots of genetic diversity, which were mainly located in the southern portion of the peninsula. Yet, our results showed that the

protected area network fail to cover most of the area hotspot (> 65 %). Although we found substantial differences in hotspot location and extent when considering mitochondrial or nuclear data, both mitochondrial and nuclear hotspots resulted not adequately protected. Finally, we estimated that both hotspot extent and its portion covered by protected areas will dramatically decline in the future, following the species distribution shifts induced by the ongoing climate changes.

4.1. How much genetic diversity is currently covered by the Italian protected area network?

For all the lineages, the estimated area of bioclimatic suitability (Zenodo, doi: https://doi.org/10.5281/zenodo.12706002) largely overlaps with the current species distribution (Sillero et al., 2014). Yet, for most of the lineages, the area of suitable range included in protected areas is <15 % (Table 1). It is worth noting that among the lineages with the lowest inclusion, there are the most threatened, *Bombina pachypus* (10 %) and *Testudo hermanni* (8 %), both included "Endangered" in the IUCN Red List and showing severe population decline caused by habitat loss and degradation (Rondinini et al., 2022). In contrast, the lineages showing higher inclusion are mainly lineages with a narrow range restricted to mountain forest habitats (*Sciurus meridionalis*, *Myodes glareolus*, *Salamandrina terdigitata*). This pattern reflects the past trend to design protected areas mainly in mountain regions (>50 % of the protected area surface in Italy is above 600 m asl; note that 32.63 % of Italy is above 600 m) and highlights the need to increase the extent of protected areas in lowland habitats.

We found a very small amount of genetic diversity included within protected areas for almost all the analyzed lineages (see Table 1 and Fig. 1). The areas of high genetic diversity (*i.e.,* single species genetic hotspot) included within protected areas is below 20 % coverage for 60 % of analyzed lineages, and below 10 % for 40 % of lineages. In the case of *B. pachypus*, we found only 6 % of its nuclear genetic diversity hotspot covered by protected areas. The situation is even worse for the southern lineage of *Lissotriton italicus*, for which no hotspots are covered by protected areas. By overlapping the areas with the higher level of genetic diversity for all the investigated lineages we obtained the hotspots of genetic diversity for non-volant terrestrial vertebrates endemic to the peninsula. Only 30 % of the nuclear hotspot and 34 % of the mitochondrial hotspot are included into the protected area system. Noteworthy, most of the hotspot area falls just outside of some existing national parks or, when inside, it is close to the boundaries and thus getting lower level of protection according to the park zonation scheme (Geneletti and van Duren, 2008). Overall, these data highlight critical danger for the studied lineages, and likely mirror a more generalized pattern across the Italian biota: the populations with the higher chance to cope with the ongoing global changes are mostly not protected, but directly exposed to the anthropogenic pressures.

We found most of the genetic diversity concentrated in the southern portion of the peninsula. Literature on phylogeographic patterns across the European region had already identified the southern portion of Mediterranean peninsulas as a hotspot of intraspecific genetic variation (Hewitt, 2011; Canestrelli et al., 2010; Schmitt et al., 2021). However, by overlapping fine-scale mapping of genetic data for single species, we obtained accurate location of multi-species hotspots at high-resolution geographic scale, with two main outcomes. First, knowing the exact location of genetic diversity hotspots allows effective integration of genetic diversity in spatial conservation planning, providing the baseline for an informed increasing of the extent of protected areas that account for the biodiversity evolutionary potential. Second, the identification of multispecies hotspots opens up a better understanding of the evolutionary processes generating them. To date, the rise of genetic diversity hotspots has been linked to long-term environmental stability of populations (Carnaval et al., 2009). In the Mediterranean region, indeed, hotspots have been for long time considered coinciding with glacial refugia (Petit et al., 2003; Stewart et al., 2010). However, growing evidence is supporting the alternative hypothesis seeing glacial refugia as dynamic and transient entities, and the arising of hotspots as the result of the underling dynamic processes. Looking at the exact location of the hotspots, we found them mainly located in lowlands surrounding mountain massifs, which correspond to areas that experienced drastic environmental changes during the Pleistocene, including overflooding during interglacial phases (Bonfiglio et al., 2002; Caloi et al., 1989; Tansi et al., 2007; Tortorici et al., 1995). Furthermore, these areas do not coincide with glacial refugia for these lineages, as they are located mainly in coastal areas currently below sea level (Chiocchio et al., 2024). Therefore, the rise of hotspots in southern Italy can be considered the result of dynamic processes including population fragmentation, short-range migration, and gene exchange among refugial populations, rather than long-term demographic stability. From a conservation perspective, integrating these hotspots into the system of protected areas will result in protecting those populations having in their genetic background the evolutionary potential to cope with dynamic environments.

4.2. Is there concordance between mitochondrial and nuclear genetic data?

Our results showed substantial discordance between the location of hotspots derived from mitochondrial and nuclear genetic data, particularly at biologically relevant spatial scales. Both mitochondrial and nuclear hotspots were located in the southernmost part of the peninsula, in the Calabrian region, but displaced in different areas. The mean overlap between the two areas is below 9 %, but for many lineages is 0 or <5 %. The overlap between the multispecies hotspot is about 3 % (165 km^2) of their cumulative surface. It is worth noting that for almost all the investigated lineages the two datasets were largely congruent, as we were able to extract both mitochondrial and nuclear data from the same location, and we applied the same interpolation procedure. Thus, such discordance reflects substantial differences between the two genomic markers in the processes shaping the distribution of genetic variation across populations. These differences may affect conservation planning and thus deserve further consideration. Indeed, mitochondrial DNA is subject to different heritability patterns, mutation rates, and selective processes as compared to the whole nuclear genome, and they are not sufficient alone for inferring processes shaping population demography and evolutionary history (Ballard and Whitlock, 2004; Hill, 2015; Zink and Barrowclough, 2008; Edwards and Bensch, 2009). For these reasons, the diversity of mitochondrial DNA is not systematically related to the genome-wide diversity (Bazin et al., 2006; Nabholz et al., 2008; Galtier et al., 2009; James and Eyre-Walker, 2020), and thus not representative of population evolutionary potential. Our results confirm unrelated diversity patterns between mitochondrial and nuclear genomes and call for caution in using mitochondrial genetic data for conservation purposes, irrespective of their higher availability. In fact, although motivated by the wider availability of mitochondrial data in public database, using maps of genetic diversity based on mitochondrial data (see *e.g.* Miraldo et al., 2016; Dapporto et al., 2022; French et al., 2023) could lead policymakers to address conservation initiatives on worthless areas and/or populations (Mazel et al., 2018; Schultz et al., 2024), dispersing the efforts and the scanty investments on biodiversity conservation. On this novel perspective, we suggest dismissing the use of mitochondrial genetic data in conservation biology, as it looks unreliable at biological relevant scale, and to promote novel, region-wide assessment of genome-wide patterns of genetic variation.

4.3. Will the current protected area network protect genetic diversity under climate change?

Our results show that the current system of protected areas is unable to protect both species and genetic diversity from the effects of climate change. By projecting species distribution to 2100, for all the lineages

but one (the northern lineage of *Hyla intermedia*) we found a substantial decrease in suitable range and areas of high genetic diversity – irrespective of the emission scenario employed. Accordingly, almost all the lineages showed a dramatic loss of both suitable area and areas of high genetic diversity included in the current system of protected areas. Notably, some lineages showed almost complete loss of representation within protected areas, including the endangered *B. pachypus*, and including some other lineages currently showing the first traces of decline: *Salamandra salamandra*, *Rana italica*, and *Salamandrina perspicillata*. Finally, under an intermediately pessimistic emission scenario, the predicted area hotspot will dramatically decline both within and outside protected areas (−48 % for mitochondrial diversity hotspot and − 25 % for nuclear diversity hotspot). These results suggest a need for a rapid re-assessment of biodiversity conservation management, both at regional and global scales.

4.4. Concluding remarks

Conservation commitments have been set to increasing the extent of protected areas through 30 % by 2030, and the importance of including genetic diversity into conservation initiatives has been raised (Kunming-Montreal Global Biodiversity Framework: Goal A and Target 3 and 4; CBD, 2022; Hoban et al., 2023). This study provides some useful insights to include genetic diversity into spatial conservation plannings. First, mitochondrial data do not reflect spatial patterns of the nuclear genome diversity, and thus their usage in spatial conservation planning might lead to ineffective initiatives. Second, we found genetic diversity of terrestrial vertebrates poorly preserved by the current system of protected areas. Yet, we identified those areas where the extension of protected areas would have the higher benefits in terms of preserving the genetic level of biodiversity under climate changes. Most of these areas are in the lowlands located among the main mountain massifs of southern Italy. These regions are mostly uncovered by protected areas. In particular, the lowland areas among the south of the Sila and the Serre mountain region and among the Aspromonte massif and the Serre region hold the main genetic diversity hotspots of the Apennine peninsula - the last being the only one persisting to the climate change. Therefore, we strongly recommend including these areas into the Italian system of protected areas. Including some of those areas requires the institution of a new area-based conservation measures. In other cases, it would be sufficient the extension of the boundaries of some existing protected areas (as in the case of the Sila and Aspromonte National Park). It is worth noting that our assessment did not consider PA zonation, but we claim to include genetic diversity hotspot into the higher level of protection.

Finally, we discourage to consider these results as fully representative of the genetic diversity patterns along the Italian region. Despite we expect similar patterns, we suspect to find many other genetic diversity hotspots revealed by similar assessments in other taxa for which there is paucity of genetic data, such as insects, freshwater organisms, or plants. Also, by not including cold-adapted taxa and not endemic species, our analysis was not able to identify putative hotspot located in the northern region, such as in the Alps surroundings. Therefore, besides spatial conservation planning initiatives, we suggest increasing the volume of genetic monitoring, in order to obtain clear assessments of the efficacy of conservation exercises on a long-term perspective, in the face of global changes.

These results can be translated into practical recommendations to implement the KMGBF Target 3 (the '30 % by 2030′ targets) and Target 4 (conserving genetic diversity in all species) at global scale. Indeed, the analytic framework applied here is not tied to the investigated area (the Italian peninsula) and taxa (terrestrial vertebrates) and can be easily replicated in other regions using virtually any taxon for which range wide data on distribution and population (nuclear) genetic diversity are available. And the availability of these data has been increasing during the last years (Leigh et al., 2024), opening for a wide use of the approach used in this study. Using the same approach to locate genetic diversity hotspots at fine geographic scale in different regions allows to standardize the procedures to define 'genetically rich' and 'climate change resilient' areas for protected area extension, thus implementing at the same time the KMGB Target 3 and 4.

CRediT authorship contribution statement

Andrea Chiocchio: Writing – review & editing, Writing – original draft, Supervision, Investigation, Formal analysis, Data curation. **Nina L. Santostasi:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Alice Pezzarossa:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Roberta Bisconti:** Writing – review & editing, Investigation, Data curation. **Luigi Maiorano:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Data curation. **Daniele Canestrelli:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

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Declaration of competing interest

The Authors declare no conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocon.2024.110828.

Data availability

The datasets generated and analysed in this study are available in the ZENODO repository at the following link doi: 10.5281/zenodo.12706002.

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