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Are we ready for applying genetic diversity indicators? A cross-taxa pilot assessment of wild animal and plant populations in Bulgaria

Violeta Zhelyazkova
violeta.lyubomirova@gmail.com
National Museum of Natural History at the Bulgarian Academy of Sciences
Maria Kachamakova
Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences
Dimitar Plachiyski
Bulgarian Society for the Protection of Birds
Salza Palpurina
National Museum of Natural History at the Bulgarian Academy of Sciences
Vera Antonova
Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences
Yordan Koshev
Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences
Viktoria Köppä
Stockholm University
Alicia Mastretta-Yanes
Royal Botanic Gardens

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Abstract

Genetic diversity ensures species adaptive potential and prospects for long-term survival; therefore, its estimation represents an essential conservation tool. For this purpose, the Kunming–Montreal Global Biodiversity Framework under the Convention of Biological Diversity endorsed a dedicated headline indicator A.4 “The proportion of populations within species with an effective population size > 500”, hereafter Ne_{500}, and a complementary indicator “the proportion of maintained populations within a species”, hereafter PM. To assess the genetic health of Bulgarian wild populations, we calculated these, using genetic or proxy data, for a set of 96 species: 36 mammals, 20 birds, 1 reptile, 2 amphibians, 5 fish, 14 invertebrates, 12 angiosperms, 5 gymnosperms, and 1 spore-bearing plant. Most of the assessed species (72%), had a favourable PM indicator value of 1, meaning they had not lost any population since the baseline year 2000. However, only 44% had a favourable value of Ne_{500}=1, meaning all their populations surpassed the threshold of an effective population size of 500 individuals. This emphasizes the need to include information on genetic diversity in the national legislation and biodiversity monitoring programs, which is currently not the case. To fill this gap and facilitate the implementation of the PM and Ne_{500} indicators in Bulgaria, we discuss their appropriate use in different taxonomic groups and outline our vision of future genetic research and monitoring in the country. Despite the use of local and non-exhaustive data, our insights and recommendations are useful for other countries where genetic monitoring is still in its infancy.

Introduction

Genetic diversity is one of the three components of biological diversity along with species and ecosystem diversity, as defined in the United Nations’ Convention of Biological Diversity (CBD). Staying at the lowest level of biological systems, upon which all next levels depend, genes determine the adaptive potential of populations and ensure (or not) their long-term survival. Therefore, the loss of genetic diversity is currently raising considerable concern among researchers and policymakers, particularly in the context of strong biodiversity loss drivers such as climate change, pollution, direct exploitation, changing land and sea use, and invasive species (as outlined by IPBES, the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services). The documented losses of genetic diversity range from 6% to 30% across a number of taxa according to large-scale empirical studies (Exposito-Alonso et al., 2022; Leigh et al., 2019). Further projections suggest that, in the absence of effective conservation measures, future losses could surpass 50% of existing genetic diversity (Hoban et al. 2021b). Such reductions would severely compromise organismal fitness, rendering many species and populations demographically or evolutionarily non-viable (Frankham 2022). These co-called “living dead” species or "ghost species" that have lost a substantial part of their numbers (Hanski et al. 1996) and genetic diversity respectively are prone to accelerated extinction processes and conceal the actual extinction rates and the urgency of the needed actions.

Despite the evident importance of genetic diversity for species viability and its potential to guide effective biodiversity conservation and restoration action, genetics is often missing in conservation policy and monitoring frameworks on national and international levels (Hoban et al., 2021a). In Europe for example, genetic diversity is not mentioned in the EU Habitat directive and is only implied within the notion of “favorable conservation status” (Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora). The Kunming–Montreal Global Biodiversity Framework (KMGBF), adopted under CBD in 2022, represents a major step filling this gap, explicitly underscoring the central role of genetic diversity in the conservation of wild species, particularly within Goal A and Target 4. CBD endorsed a dedicated headline indicator A.4 “The proportion of populations within species with an effective population size > 500”, hereafter Ne_{500}, and a complementary indicator “the proportion of maintained populations within a species”, hereafter PM (CBD, 2022). Member countries were invited to report these for the first time in February 2026.

In simple terms, effective population size (*Ne*) is the population size that governs evolutionary processes and determines the rate of genetic drift, inbreeding and variance in the next generations (Waples 2022). An effective population size of minimum 500 (*Ne* > 500) was first proposed by Franklin (1980). Based on his work with *Drosophila* flies, Franklin (1980) calculated that *Ne* > 500 was necessary to balance the random loss of genetic variation (though genetic drift) with the accumulation of mutation-driven gains. Note that due to multiple factors such as unequal sex-ratio or unequal reproductive success, *Ne* is usually much smaller than the actual (census) population size (*Nc*). Although great variations exist between and even within species, an average

Ne/*Nc* ratio of 0.1 was first proposed by Frankham (1995) and is nowadays widely accepted within the scientific community as a somewhat conservative measure that would be applicable to most species. Based on this ratio, *Nc* > 5000 roughly corresponds to *Ne* > 500, which is in turn considered large enough to maintain evolutionary potential in perpetuity (Jamieson and Allendorf 2012; Hoban et al. 2021). *Nc* > 5000 is also close to the minimum viable population size of 5500 that was estimated for various groups (birds, mammals, and plants) by Thomas (1990) based on extinctions reported from wild populations. The proportion of maintained populations, on the other hand, is related to the older "comprehensiveness" CBD indicator, focused on conserving species' geographic range. It emphasizes the importance of genetic distinctiveness that can exist between populations of the same species and its crucial role in local adaptation. In order to properly assess and monitor species genetic health, it is recommended that the two genetic diversity indicators, Ne_{500} and PM, are used together (Hoban et al. 2020, Hoban et al. 2023b). Additionally, a third indicator "the number of species within a country that are being genetically monitored" was proposed (Hoban et al. 2020) that was not officially adopted by CBD but is useful for assessing the progress in a county's efforts toward studying and preserving genetic diversity. Importantly, both Ne_{500} and PM can be calculated with or without genetic data, extracting information from already established species monitoring schemes and published databases, which makes them easily applicable even in countries with limited resources for genetic research (Mastretta-Yanes et al. 2024).

Assessing genetic health of populations is particularly relevant in the Balkan Peninsula, including Bulgaria as the region is widely recognised as a major Pleistocene refugium and a center of endemism. This biogeographic history suggests that many taxa are likely to retain high levels of genetic diversity and adaptive potential (Koshev et al. 2023; Kryštufek 2004; Temple & Terry 2007; Ricanova et al. 2013; Borowiec 2014; Golemansky & Popov 2015; Rossiter et al. 2007; Bilgin et al. 2008; Rebelo et al. 2012; Gömöry et al. 2020), with genetically distinct populations of multiple species such as those of Martino's mole *Talpa martinoirum* (Kryštufek et al. 2018, Demirtaş et al. 2022), Capercaillie (*Tetrao urogallus*) (Duriez et al. 2007; Bajc et al. 2011; Klinga et al. 2015, Escoda et al. 2024), Rock partridge (*Alectoris graeca*) (Randi et al. 2003), or Northern chamois (*Rupicapra rupicapra*) (Anderwald et al. 2020, Rezić et al. 2022). Yet, the present study is the first one to evaluate the state of genetic diversity preservation and the long-term adaptive potential of Bulgarian wild species. We do this by calculating the Ne_{500} and the PM genetic diversity indicators adopted by CBD, using available genetic and non-genetic data. We then discuss the applicability of these indicators in national biodiversity monitoring frameworks and species conservation status assessments in different taxonomic groups and we outline our vision of future genetic research and monitoring in the country. Results were used to support the country's 7th National Report to CBD in early 2026.

Methods

Data collection and analysis

For data collection, we used the KoboToolBox (https://www.kobotoolbox.org/) to implement a questionnaire developed by Alicia Mastretta-Yanes and Viktoria Köppä as a part of the COST Action CA23121 - Genetic Nature Observation and Action (GENOA). The same tool was used to collect data on genetic diversity indicators for various taxa across 18 countries in Europe (publication in prep.). Questions were designed to collect data on: (1) the number of extant and extinct populations per assessed species, (2) estimated population census size (*Nc)*, reported either as a point or range count; (3) effective population size (*Ne*), and (4) any genetic studies available. Metadata included information about species geographical range and habitat, national and international conservation status and legal protection, and the presence of any transboundary populations. The resulting dataset was downloaded as a .csv file and processed in R v.4.3.2 (R Core Team 2023), where we filtered only taxa for Bulgaria. For quality check and indicator calculation, we used the code provided in https://github.com/AliciaMstt/GeneticIndicators, with custom optimizations. Several taxa were evaluated at the subspecies level; however, for clarity and consistency, all taxa were treated as species.

Calculation of genetic diversity indicators

For the Ne_{500} indicator, we either took *Ne* values directly from the available studies, or we converted *Nc* to *Ne* data using the standard *Ne*/*Nc* ratio of 0.1, when only Nc information was available. To make mathematical operations possible, we converted

Nc range data to point data as described in Mastretta-Yanes (2024). Briefly, populations noted as having slightly more than 5000 individuals were allocated a census size of 5500; populations noted as being substantially larger than 5000 individuals were assigned a value of 10,000; populations noted to have a census size of just under 5000, were assigned a value of 4500; and populations with considerably fewer than 5000 individuals were given a value of 500. Finally, we calculated Ne_{500} for each species by dividing the number of populations with *Ne* > 500 by the total number of populations. For the PM indicator, the year 2000 was taken as the baseline and calculations were made according to the following equation: PM = number of extant populations in 2026/(number of extant populations in 2026 + number of extinct populations since 2000). To delineate populations, we used the "subpopulation" definition as provided in the IUCN Red List Guidelines (IUCN 2001): "geographically or otherwise distinct groups between which there is little demographic or genetic exchange, typically less than one successful migrant individual or gamete per year". In the Kobo toolbox questionnaire, we also specified whether we delimited species populations based on one or more of the following criteria: geographical boundaries, dispersal buffers, ecological or biogeographic proxies, adaptive traits, genetic clusters/clades, management units, or a combination of those. For the additional genetic monitoring indicator, we also summarised information about genetic monitoring (genetic diversity studies using DNA-based methods repeated in time) or other genetic studies (e.g., phylogenetic or population genetics). The raw data is provided in Supplement 1 and the R script and the custom functions used for the analysis are in Supplements 2 to 5. Data on the national conservation status of the assessed species is present in Supplement 7.

Results

Data availability

We filled in the questionnaire in KOBO toolbox for 96 species: 36 mammals, 20 birds, 1 reptile, 2 amphibians, 5 fish, 14 invertebrates, 12 angiosperms, 5 gymnosperms, and 1 pteridophyte. For some taxonomic groups, species choice was restricted mainly by data availability: for example, the 13 species of bats that we included in the study represent all bat species for which any population size information is present in the country. For other taxonomic groups, species choice was more restricted by the short timeframe of the project or data accessibility. For example, plants selected for this study were mostly those with population data readily available in Species Action Plans (SAPs; https://www.moew.government.bg/bg/priroda/biologichno-raznoobrazie/zastiteni-vidove/planove-za-dejstvie/; accessed: 3rd July 2026), however, not all such plans were analysed. Additionally, not all data available at the National System for Monitoring of the Status of Biodiversity (NSMSB) was utilised as it requires an *apriori* request to access the data (ExEA, 2026), which brings further time restrictions.

Overview of the assessed populations

Within the analysed species, population size values (*Nc*) were mostly given as ranges (e.g. "a few hundred", 177 out of 253 or 70% of assessed populations and 74% of assessed species), whereas point values were available only for some mammals, birds, and angiosperm plants (60 out of 253 or 24% of assessed populations and 27% of assessed species); for 16 populations census size was unknown (Fig. 1, "Census population size"). Most of the assessed species were reported as having just one or two populations (n = 70), with only 3 species having more than 10 populations (the orchid *Dactylorhiza incarnata* and the amphibians *Triturus cristatus* and *Mesotriton alpestris*) (Fig. 1, "Number of populations"). The majority of species (n = 61) were also reported to have at least one transboundary population, with a total of 79 transboundary populations (Fig. 1, "Transboundary populations"). Not surprisingly, smaller and less mobile species had a larger number of populations, which were more often restricted to the country in comparison to larger and more mobile species (e.g., birds and mammals), which more often had one or a few transboundary populations. Effective population size data was available only for 3 species, all of which were mammals: wolf *(Canis lupus*) (Moura et al. 2014, for Bulgaria), Black Sea bottle-nosed dolphin (*Tursiops truncatus ponticus*) (Viaud-Martinez et al. 2008, for the entire Black Sea population), and Black Sea harbour porpoise (*Phocoena phocoena relicta*) (Fontaine et al. 2012, for the entire Black Sea population) (Fig. 1, "Effective population size data"). This data is presented for each population in Supplement 6.

Genetic diversity indicators and conservation status

According to the Ne_{500} indicator, 42 (44%) of the assessed species have maintained favorable population sizes (Ne_{500} = 1) across all populations, however 41 (43%) did not have even one population with *Ne* > 500 (Ne_{500} = 0) (Table 1; Fig. 1, "Ne_{500} value". According to the PM indicator, most species (n = 69, 72%) have not lost any populations since the year 2000 (PM = 1) and only one species was reported extinct (*Cricetus cricetus*, PM = 0). Yet, PM was not estimated for 18 species (19%) due to the lack of precise information on extinct populations (Table 1; Fig. 1, "PM value"). Census size data for each population is presented in Supplement 6. Genetic monitoring has been conducted in the brown bear, *Ursus arctos* (MOEW, 2025), mainly in relation to human-wildlife conflicts, and partly for the invertebrate *Rosalia alpina* (Drag et al. 2015) (Fig. 1, "Genetic monitoring conducted"). Some genetic research (e.g., phylogenetic or population genetic) has been conducted in Bulgaria in 49 (51%) species from all taxonomic groups besides reptiles and pteridophytes, with the proportion of studied species being highest in mammals (n = 27) (Fig. 1, "Genetic studies conducted").

Table 1

Genetic diversity indicator values and conservation status of all assessed species. ExtantP = number of extant populations by the end of 2025; ExtinctP = number of extinct populations since 2000; PopDef = population definition method, where AT = adaptive traits, DB = dispersal buffer, EBP = ecobiogeographical proxies, GB = geographic boundaries, GC = genetic clusters, MU = management units, and O = other; Transb = presence of transboundary populations; BigPops = number of populations with effective population size *Ne* > 500 (corresponding to census population size *Nc* > 5000); Ne = Ne_{500} indicator (proportion of populations with *Ne* > 500); PM = PM indicator (proportion of preserved populations since 2000); IUCN = conservation status according to the International Union for the Conservation of Nature; EUDir = presence in the European Habitat Directive (abbreviated HD) or Bird Directive (abbreviated BD), numbers refer to the annexes where the species is mentioned; RedBook = national conservation status according to the Bulgarian Red Book; BDA = presence in the Bulgarian Biodiversity Act, where numbers refer to the annexes where the species is mentioned.

Taxon	ExtantP	ExtinctP	PopDef	Transb	BigPops	Ne	PM	IUCN	EUDir	RedBook	BDA
Mammals											
Barbastella barbastellus	1	0	AT GC MU	Yes	1	1	1	NT	HD, 2 & 4	VU	2 & 3
Canis aureus	1	0	GC	Yes	1	1	1	LC	-	-	4
Canis lupus	1	0	GB GC O	Yes	0	0	1	VU	HD, 5	LC	2 & 4
Capreolus capreolus	1	0	GC	Yes	1	1	1	LC	-	-	-
Cervus elaphus	2	0	GB GC	Yes	1	0.5	1	LC	-	-	-
Chionomys syriacus aleco	7	0	GB GC	Yes	0	0	1	LC	-	-	-
Cricetus cricetus	0	1	GB	No	-	-	0	CR	HD, 4	VU	3
Delphinus delphis ponticus	1	0	MU	Yes	1	1	1	-	HD, 2 & 4	-	-
Eri-ceus roumanicus	1	0	GC	Yes	1	1	1	LC	-	-	-
Felis sylvestris	1	0	GC	Yes	0	0	1	LC	HD, 2 & 4	EN	-
Lepus europaeus	1	0	GC	Yes	1	1	1	LC	-	-	-
Lutra lutra	1	0	GC	Yes	0	0	1	NT	HD, 2 & 4	VU	2
Martes foina	1	0	GC	Yes	1	1	1	LC	-	-	-
Mesocricetus newtoni	2	1	GB	-	0	0	0.67	VU	HD, 2 & 4	VU	2 & 3
Miniopterus schreibersii	1	0	AT GC MU	Yes	1	1	1	VU	HD, 2 & 4	VU	2 & 3
Mustela nivalis	1	0	GC	Yes	1	1	1	LC	-	-	3
Myomimus roachi	3	0	GB	Yes	0	0	1	VU	HD, 4	VU	2 & 3
Myotis bechsteinii	1	0	AT GC MU	Yes	1	1	1	NT	HD, 2 & 4	VU	2 & 3
Myotis blythii	1	0	AT GC MU	Yes	1	1	1	LC	HD, 2 & 4	NT	2 & 3
Myotis capaccinii	1	0	AT GC MU	Yes	1	1	1	VU	HD, 2 & 4	VU	2 & 3

Taxon	ExtantP	ExtinctP	PopDef	Transb	BigPops	Ne	PM	IUCN	EUDir	RedBook	BDA
Mammals											
Myotis emarginatus	1	0	AT GC MU	Yes	0	0	1	LC	HD, 2 & 4	VU	2 & 3
Myotis myotis	1	0	AT GC MU	Yes	1	1	1	LC	HD, 2 & 4	NT	2 & 3
Nyctalus noctula	1	0	AT MU	Yes	1	1	1	LC	HD, 4	LC	3
Phocoena phocoena relicta	1	0	MU	Yes	1	1	1	VU	HD, 2 & 4	VU	2 & 3
Rhinolophus blasii	1	0	AT MU	Yes	0	0	1	LC	HD, 2 & 4	VU	2 & 3
Rhinolophus euryale	1	0	AT MU	Yes	1	1	1	NT	HD, 2 & 4	VU	2 & 3
Rhinolophus ferrumequinum	1	0	AT GC MU	Yes	1	1	1	LC	HD, 2 & 4	NT	2 & 3
Rhinolophus hipposideros	1	0	AT GC MU	Yes	1	1	1	LC	HD, 2 & 4	LC	2 & 3
Rhinolophus mehelyi	1	0	AT MU	Yes	1	1	1	VU	HD, 2 & 4	VU	2 & 3
Rupicapra rupicapra balcanica	5	0	GB MU	No	0	0	1	LC	HD, 2 & 4	EN	2 & 3
Sciurus vulgaris balcanicus	1	0	AT	Yes	1	1	1	LC	-	-	-
Sus scrofa	2	0	GC	Yes	2	1	1	LC	-	-	-
Talpa europaea	1	0	GC	Yes	1	1	1	LC	-	-	-
Talpa martinorum	1	0	GB GC	Yes	0	0	1	LC	-	-	-
Tursiops truncatus ponticus	1	0	MU	Yes	1	1	1	LC	HD, 2 & 4	VU	2
Ursus arctos	1	0	GB GC O	Yes	0	0	1	LC	HD, 2	EN	2
Birds											
Aegolius funereus	3	0	GB DB EBP MU	Yes	0	0	1	LC	BD, 1	VU	2 & 3
Aegypius mochus	1	0	GB EBP MU	Yes	0	0	1	NT	BD, 1	RE	2 & 3
Alectoris graeca graeca	1	0	GB MU	Yes	0	0	1	NT	BD, 1 & 2	EN	2 & 4
Aquila chrysaetos	1	0	DB EBP MU	Yes	0	0	1	LC	BD, A 1	VU	A 2 & 3

Taxon	ExtantP	ExtinctP	PopDef	Transb	BigPops	Ne	PM	IUCN	EUDir	RedBook	BDA
Mammals											
Aquila heliaca	1	0	GC MU O	Yes	0	0	1	VU	BD, A 1	CR	A 2 & 3
Athene noctua	1	0	GC MU	Yes	1	1	1	LC	-	-	A 3
Ciconia ciconia	1	0	MU	Yes	1	1	1	LC	BD, A 1	-	A 2 & 3
Clanga pomari-	1	0	GB GC MU	Yes	0	0	1	LC	BD, A 1	VU	A 2 & 3
Coturnix coturnix	1	0	DB MU	Yes	1	1	1	LC	BD, A 2	-	A 4
Crex crex	1	0	EBP MU	Yes	1	1	1	LC	BD, A 1	VU	A 2 & 3
Dendrocopos leucotos lilfordi	4	0	GB DB EBP MU	Yes	0	0	1	LC	BD, A 1	EN	A 2 & 3
Falco naumanni	1	1	GC MU	Yes	0	0	0.5	LC	BD, A 1	CR	A 2 & 3
Glareola pratincola	2	0	DB EBP MU	Yes	0	0	1	LC	BD, A 1	EN	A 2 & 3
Glaucidium passerinum	2	0	DB EBP MU	Yes	0	0	1	LC	BD, A 1	EN	A 2 & 3
Gyps fulvus	1	0	DB EBP MU	Yes	0	0	1	LC	-	EN	A 2 & 3
Neophron percnopterus	1	0	DB EBP MU	Yes	0	0	1	EN	BD, A 1	EN	A 2 & 3
Picoides tridactylus alpinus	1	0	DB EBP MU	Yes	0	0	1	LC	BD, A 1	EN	A 2 & 3
Streptopelia turtur	1	0	MU	Yes	1	1	1	VU	BD, A 2	-	A 4
Tetrao urogallus rudolfi	2	1	DB EBP MU	Yes	0	0	0.67	LC	BD, 1, 2 & 3	EN	A 2, 4 & 6
Tetrastes bonasia	4	0	DB EBP MU	Yes	1	0.25	1	LC	BD, A 1	-	A 2 & 3
Reptiles											
Ophisops elegans	3	-	GB DB	Yes	0	0	-	LC	HD, A 4	VU	A 3
Amphibians											
Mesotriton alpestris	11	-	GB DB	Yes	3	0.27		LC	-	VU	-
Triturus cristatus	23	1	GB DB	-	0	0	0.96	VU	HD, A 2 & 4	LC	A 3

Taxon	ExtantP	ExtinctP	PopDef	Transb	BigPops	Ne	PM	IUCN	EUDir	RedBook	BDA
Mammals											
Fish											
Alburnus mandrensis	1	0	GB	No	-	-	1	EN	-	-	-
Alosa pontica	1	0	GB	Yes	1	1	1	LC	HD, A 2 & 5	VU	A 4
Cottus gobio	6	-	GB	No	2	0.33	-	LC	HD, A 2	CR	A 2
Cottus haemusi	2	-	GB	No	0	0	-	EN	-	-	-
Gobio kovatschevi	2	0	GB	No	-	-	1	LC	-	-	-
Invertebrates											
Anisus vorticulus	2	-	GB EBP	No	2	1	-	DD	HD, A 2 & 4	NT	A 2 & 3
Carabus hungaricus	1	-	GB EBP	No	1	1	-	-	HD, A 2 & 4	-	A 2 & 3
Coenagrion ornatum	3	0	GB EBP	Yes	3	1	1	LC	HD, A 2	LC	A 2
Euplagia quadripunctaria	1	0	EBPMU	Yes	1	1	1	-	HD, A 2	-	-
Formica lugubris	8	-	GB DB	Yes	2	0.25	-	NT	-	-	-
Formica pratensis	8	-	GB DB	-	0	0	-	NT	-	-	-
Formica rufa	9	-	GB DB	Yes	0	0	-	NT	-	-	A 3
Lucanus cervus	1	-	GB EBP	-	1	1	-	NT	HD, A 2	NT	A 2 & 3
Lycaena dispar	1	-	GB EBP	-	1	1	-	NT	HD, A 2 & 4	LC	A 2 & 3
Nymphalis vaualbum	2	1	EBP	Yes	0	0	0.67	LC	HD, A 2	-	A 2 & 3
Paracaloptenus caloptenoides caloptenoides	5	-	GB DB	Yes	3	0.6	-	NT	HD, A 2 & 4	NT	A 2 & 3
Polyommatus eros eroides	1	-	GB EBP	-	1	1	-	-	HD, A 2 & 4	LC	-
Rosalia alpina	1	-	GB EBP	-	1	1	-	VU	HD, A 2 & 4	LC	A 2 & 3
Theodoxus transversalis	1	-	GB EBP	-	0	0	-	EN	HD, A 2 & 4	-	A 2 & 3
Angiosperms											
Aldrovanda vesiculosa	2	0	GB	No	0	0	1	EN	HD, A 2	CR	A 2 & 3

Taxon	ExtantP	ExtinctP	PopDef	Transb	BigPops	Ne	PM	IUCN	EUDir	RedBook	BDA
Mammals											
Castanea sativa	8	0	GB DB GC	Yes	1	0.12	1	LC	-	EN	-
Convolvulus persicus	1	4	DB GC	No	0	0	0.2	-	-	CR	A 3
Crambe tataria	4	1	GB DB EBP	No	0	0	0.8	-	HD, A 2	EN	A 2 & 3
Cypripedium calceolus	2	0	GB	No	0	0	1	LC	HD, A 2 & 4	CR	A 2 & 3
Dactylorhiza incarnata	25	-	GB	No	0	0	-	LC	HD, A 2	EN	A 3
Marsilea quadrifolia	1	3	DB	No	0	0	0.25	LC	HD, A 2 & 4	CR	A 2 & 3
Orchis provincialis	4	0	GB DB	No	0	0	1	LC	-	CR	A 3
Platanus orientalis	4	0	DB	-	2	0.5	1	VU	-	-	-
Primula deorum	6	0	GB	No	2	0.33	1	DD	HD, A 2	VU	A 3
Ramonda serbica	5	-	DB GC	No	0	0	-	LC	-	EN	A 3
Stipa ucrainica	4	0	DB	No	0	0	1	-	-	CR	-
Gymnosperms											
Abies alba	1	0	GC MU	-	1	1	1	LC	-	-	-
Pinus mugo	1	0	GC MU	Yes	1	1	1	LC	-	-	-
Pinus nigra	4	0	GC	-	4	1	1	LC	-	-	-
Pinus peuce	1	0	GC MU	-	1	1	1	LC	-	-	-
Pinus sylvestris	2	0	GB EBP GC	Yes	2	1	1	LC	-	LC	-
Pteridophytes											
Isoetes pirinica	7	-	GB	No	1	0.14	-	-	-	EN	-

As for the national conservation status, out of the 96 assessed species, 33 were not listed in the Bulgarian Red Book, 9 were of least concern, 6 were near threatened, 22 were vulnerable, 16 were endangered, 9 were critically endangered and 1 was regionally extinct. Importantly, there were 19 species that were either not listed in the Red Book or listed as least concern, where the Ne_{500} and/or PM indicator had unfavourable values (< 1) or could not be calculated. Additionally, there were 13 species with favourable values of both indicators (= 1) that were listed in the Red Book as vulnerable or near threatened (Table 1; Fig. 2; Supplement 8).

Discussion

The present study is the first in Bulgaria to evaluate the two CBD genetic diversity indicators, Ne_{500} for effective population size and PM for the proportion of maintained populations, adopted by CBD. Due to gaps in data availability and the narrow project time frame, our dataset is not exhaustive and cannot be viewed as a systematic review for all taxonomic groups in the country.

Yet, it provides some valuable insights on the indicators' applicability at the national level and identifies important knowledge gaps and research priorities for the future.

Genetic diversity indicators' applicability

We calculated the Ne_{500} indicator for 93 and the PM indicator for 78 out of the 96 assessed species. As demonstrated by Mastretta-Yanes (2024), even in the absence of actual genetic information, calculating the indicators was fairly straightforward. Yet, information on the number of extinct populations (which prevented calculating the PM indicator) was missing for 18 species. This emphasizes the risk of genetic variants going extinct unnoticed (e.g., Allendorf 2024), particularly in smaller and less mobile species with a high number of restricted populations that are hard to find and monitor. Of note, in the absence of detailed genetic research, delineating populations is by itself a challenge that inevitably involves some assumptions. For example, for many mammal and bird species in our study, we defined a single national population using the following: 1) large-scale genetic studies showing weak population structure of the particular species across Europe, yet sometimes with just a few or no samples from Bulgaria; 2) certain morphological traits (e.g., the ability to fly or a large effective pollination distance) allowing high dispersal capacity (for birds and bats), or 3) high mobility combined with even distribution across the country (e.g. middle-sized mammals). While such assumptions are sound and probably valid in most cases, contradicting data exist. For example, using SNP genotyping, Medinas et al. (2023) found that fine-scale population structure is present in lesser horseshoe bats (*Rhinolophus hipposideros*) in Portugal and that human-made structures such as roads represent a barrier to gene flow. Based on the assumptions we would assign one population of wild boar (*Sus scrofa*), but the genetics show that this is not the case (Nikolov at al. 2009). Regarding effective pollination distance, wind-pollinated plants such as the white pine *Pinus sylvestris* were good candidates to exchange genes between distant populations but too many parameters play in hand such as topography or prevailing wind direction, which can limit effective pollination. Therefore, we decided to divide this species into populations based on well-defined mountain ranges.

As for the Ne_{500} indicator, we managed to estimate it for all but 3 species, however, most of the calculations (n = 90) were based on the conversion of census size to effective population size data and not on direct estimates of *Ne*. This means that resulting *Ne* values are only approximate as great variations from the standardly used *Ne*/*Nc* ratio exist (Frankham 1995), even within the same species. For example, for the bat *Myotis bechsteinii*, Wright et al. (2021) calculated an *Ne/Nc* ratio of 0.78 for a population of the size of 30 bats, and of 0.24 for a population of 330 bats. Furthermore, clonal reproduction that is common in social insects and in many plant species significantly reduces *Ne* (Gargiulo et al. 2023: the clonal orchid *Cypripedium calceolus*), sometimes with orders of magnitude, yet, large clonal populations can be dominated by a few successful genotypes without suffering negative fitness consequences (Young et al., 1996; Wójkiewicz et al., 2021).

Nevertheless, and despite all of the abovementioned limitations, we agree that the adoption of simple standard indicators for the assessments of species genetic diversity is useful and could serve as a good starting point for more precise definitions of research and conservation priorities.

Genetic diversity indicator values

Most of the assessed species (n = 69) had favourable values of the PM indicator of 1, indicating the positive tendency of preserving all their populations since 2000. Yet, our dataset is biased towards the more mobile mammals and birds with just one or two populations in the country, which face smaller risks of losing a whole population in comparison to smaller and less mobile species with numerous distinct populations. A significantly more negative trend appears according to the Ne_{500} indicator where 42 of the assessed species had favourable Ne_{500} values of 1, but 41 species had the highly unfavourable value of 0. In some cases, this may not be a cause of immediate concern as a big proportion of the discussed populations were transboundary (Table 1), meaning that their actual size should be bigger that the size described by this study. Additionally, there are species, such as many vascular plants, that naturally occur as small, fragmented populations of only a few individuals and may appear extinct during prolonged underground dormancies (see e.g. the orchid *Hammarbya paludosa*; Tatarenko et al. 2022), without necessarily experiencing a loss of genetic diversity (Young et al. 1996). Yet, in order to prevent decline in populations' genetic health before it is too late, we recommend that more research attention is given to species with Ne_{500} values < 1 and that these

values are reflected in species national conservation status. Last but not least, we need to keep in mind that in healthy ecosystems, some species, such as many bats, exist in very high numbers. For example, the bat *M. capaccinii* has a current national population size of a little more than 5000 individuals, which has led to the calculation of a favourable Ne_{500}, yet this represents a decline in comparison to the monitoring period of 2010–2012 when more than 20 000 individuals were counted (Petrov and Terziyski 2013). Importantly, high population densities are required for keystone species to perform their ecosystem services (e.g., Winfree et al. 2015) such as pollination, seed dispersal, pest control, carbon fixation, etc. Thus, favourable values of the Ne_{500} indicator should be always considered alongside temporal trends in population census size and other parameters and must not be misinterpreted as a rationale for allowing population decline in abundant species without assessing further ecological consequences.

Research recommendations

Despite the fact that our study is not a complete systematic review of all available data, it revealed several knowledge gaps that we consider valid and important to fill. First, more research attention should be directed to animal species different from birds and mammals where even basic population information is mostly lacking. Very often these are less mobile species with multiple distinct populations, which could easily be lost unnoticed. This problem is partly due to the fact that monitoring, scientific and conservation programs (e.g., the National System for Monitoring the State of Biodiversity, NSMSB) in the country are mainly concentrated within protected areas and usually cover only a subset of known populations.

Once basic population information is gathered, genetic studies should be conducted on selected high-priority species that properly discriminate populations, identify potentially isolated populations or populations with exceptionally high genetic diversity, map specific features in the landscape that influence gene flow and identify genetic exchange corridors. In order to provide the needed resolution, these studies should involve a large number of local samples in contrast to previous research that was conducted at a European level and included only a few Bulgarian samples (e.g., for *Glis glis* (Michaux et al. 2019), *Dryomys nitedula* (Çolak et al 2025 with one sample from Bulgaria); *Meles meles* (Marmi at al. 2006), Hermann's Tortoise (Perez et al. 2014); *Theodoxus transversalis* (Feher et al. 2012, with one sample from Bulgaria; Menabit et al. 2022); or ants from *Formica rufa* group (Masoni et al. 2022, Pamilo and Kulmuni 2022) or primarily address historical bottlenecks or comparative population structure (e.g. for plants: Lusini et al., 2014; Petrova et al., 2014, 2017; Naydenov et al., 2015). Special attention should be given to discovering and preserving new cryptic species and better study cryptic species that are already known but cannot be discriminated using traditional non-genetic methods. An example of the first type appears from the newest genetic study on the alpine newt (*Mesotriton alpestris*) suggesting that its population on Vlasina Plateau on the border between Serbia and Bulgaria represents a distinct species (Koster et al. 2026). An example of the second type are the three species of medium horseshoe bats (*Rhinolophus mehelyi*, *Rh. euryale*, and *Rh. blasii*): these often form mixed colonies where population size is traditionally estimated for the whole species group and not for each of the individual species, thus missing important species-specific tendencies. Lastly, some calibration of the proxy genetic indicators (e.g., comparing effective population size estimated using genetic methods or using census population size or occupied territory) and the *Ne/Nc* conversion ratio should be performed, particularly for clonal species that have markedly different population structure from the sexually reproducing ones.

The third step will be to establish standardized genetic monitoring, which does not currently exist in Bulgaria. The only step in this direction has been an assay for determination of the population numbers of the brown bear (*Ursus arctos*) through genetic analysis performed in 2022 and 2025 by the Ministry of the Environment and Water (MOEW); however, results are still not available. The reason that drove this effort was the human-bear conflict and the highly contrasting opinions of stakeholders on the bear numbers and the need for lethal population control (Ganchev at al. 2026). Of course, regular genetic monitoring is costly and time-consuming and would not be feasible for all species that are currently monitored using traditional methods; thus, a subset of species should be selected (Hvilsom et al. 2022). Ideal candidates should exhibit high site fidelity or philopatry, present a high feasibility for non-invasive genetic sampling, and possess both an established reference genome and standardized molecular markers. According to the authors' expertise, examples of species matching these criteria include the Capercaillie (*Tetrao urogallus*), Hazel grouse (*Tetrastes bonasia*), Spur-thighed tortoise (*Testudo graeca*), Hermann's tortoise (*Testudo hermanni*), Roach's mouse-tailed dormouse (*Myomimus roachi*), the Greater Horseshoe bat (*Rhinolophus ferrumequinum*) or the Greater Moused-eared bat (*Myotis myotis*). Among the invertebrates, wood ants (e.g. the *Formica rufa* group) are perhaps the closest candidate for such monitoring in our country: the genus is an established genetic model; ant mounds are permanent

structures that function in the same location for decades; colonies consist of tens of thousands to millions of workers, and collecting a few dozen individuals for population-genetic analysis has zero adverse effect on colony survival.

Conservation recommendations

Results from all of the abovementioned research would improve species conservation and management in Bulgaria by better assessing the health and long-term survival perspectives of populations. An example of particular conservation action that is informed by genetic data is preserving population connectivity or strictly protecting populations with particularly high or unique genetic diversity. Genetic data is also crucial for the success of population reinforcement and reintroduction: from identifying the need of a translocation, through the choice of donor populations based on genetic similarity, diversity and abundance, to the assessment of the action outcome.

A particularly important aspect of putting genetic knowledge into practice is the update of relevant legislation, conservation status assessments, and monitoring programs. This need is evident from this study where we documented 19 species that were either not listed in the Bulgarian Red Book or listed as least concern, but had unfavourable or unknown values of the Ne_{500} and PM indicators. This reflects the fact that genetic diversity is still not properly taken into account in species conservation assessments and that in general, the Bulgarian Red Book is updated far too slowly. So far, it has been published twice : 1st edition in 1984–1985; and 2nd edition in 2011 (electronic) and 2015 (on paper). Thus, we propose the establishment of a permanent Bulgarian Red Data Book Committee, similar to the one of IUCN, which would incorporate new species and indicators (in this case, the two genetic diversity indicators Ne_{500} and PM), change statuses, and make other updates whenever new scientific data, including genetic, become available. The Bulgarian Red Data Book should be primarily electronic, allowing easier and faster updates that should be then reflected into the species legal status according to the Bulgarian Biodiversity Act (for now, Annexes 2 and 3 of this law, where protected species are listed, have not been updated since the adoption of the law in 2002).

Next, the estimation of the Ne_{500} and PM indicators should be incorporated into the NSMSB, where their non-genetic proxies could be used for all species and detailed genetic research and monitoring could be conducted for selected high-priority species. This data should be then integrated into species action plans (SAPs) that are required only for species listed in Annex II of the Bulgarian Biodiversity Act; this gives one more reason for the law to be updated. Of note, in many national monitoring and conservation programs, species choice is guided directly by the European Habitat and Bird Directives (Council Directives 92/43/EEC and 2009/147/EC), yet in our study there are 7 species listed as endangered or critically endangered in Bulgaria but not present in the directives (Supplement 8). Such discrepancies should be taken into account and both national and European conservation statuses should be used to guide species choice for research and conservation management. On the other hand, we do not think that favourable values of the Ne_{500} and PM indicators alone are a reason for lowering the conservation category of a species as many other factors exist that can influence species vulnerability.

Additionally, special attention should be given to transboundary populations, which are present in the majority of species in our study. In such situations, as for migratory species, conservation responsibility is shared between several countries that should work together to achieve conservation success. The European Union is making progress in that direction through the establishment of the Ecological network Natura 2000 and the adoption of specific Action plans for the conservation of the brown bear and the wolf in Europe (Swenson 2000, Boitani 2000). On a regional level, similar efforts have been made in the Carpathians (Papp et al. 2020) and between Bulgaria and neighboring countries (e.g. through LIFE, Interreg and DRP projects), yet, this is very far from covering all transboundary populations. To facilitate progress, an appropriate solution would be to make national data on populations' size and geographical range publicly available in a unified format and language so that it could be easily downloaded and used by neighboring countries. On the other hand, small populations that are not transboundary require attention as well as no help for their conservation would come from elsewhere.

Last but not least, we recommend that Bulgaria continues elaborating its reports of the Ne_{500} and PM indicators as well as its progress in genetic research and monitoring in wild species to CBD, bringing its contribution to global conservation policies. For example, the 7th national report of the country to CBD, submitted in early 2026, for the first time contained information on the level of species genetic diversity preservation. Yet, this was just a short summary of the present research containing the

proportion of species with favourable or unfavourable Ne_{500} or PM values and the A.4 Indicator was not reported for individual species – a gap that we hope to fill till the next reporting period. At a European level, a new and important instrument for the achievement of CBD goals is the Nature Restoration Regulation (NRR), which entered into force in 2024. The main objective of NRR is to restore all degraded ecosystems on the EU's territory by 2050 and by September 2026 member countries should prepare their national version of the regulation, including details on which ecosystems should be restored and how. Genetic diversity is not explicitly mentioned in NRR, yet in order for biological systems to respond to conservation measures and to recover once a particular stressor has been removed, a certain degree of genetic variation and hence adaptive potential is required. Thus, we strongly advise that genetic diversity preservation is accounted for in the Bulgarian NRR and that genetic research and monitoring is listed as one of the possible measures for nature restoration and for following the progress on other restoration actions.

Declarations

Competing interests

The authors have no competing interests to declare.

Author Contribution

VK and AMY designed and distributed the data collection form. MK coordinated data collection for Bulgaria. VZ, MK, DP, SP, VA, and YK filled-in Bulgarian species data and provided the species-specific knowledge needed for data interpretation. VZ performed the data analysis, partly using the script by AMY. VZ and MK wrote the manuscript with input from all authors.

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Data Availability

All data supporting the findings of this study are available within the paper and its Supplementary Information.

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