Multinational evaluation of genetic diversity indicators for the Kunming-Montreal Global Biodiversity Monitoring framework


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Mastretta-Yanes, da Silva, et al. (pre-print)

Abstract

In December 2022, the United Nations Convention on Biological Diversity (CBD) adopted the Kunming-Montreal Global Biodiversity Framework, in which 196 Parties, for the first time, committed to report on the status of genetic diversity for all species. To facilitate this reporting, three genetic diversity indicators were developed, two of which focus on the processes contributing to genetic diversity loss: the loss of genetically distinct populations (measured by complementary indicator “proportion of populations maintained within species”) and populations being too small to maintain genetic diversity (measured by headline indicator A4, “The proportion of populations within species with an effective population size > 500”). The major advantage of these indicators is that they can be estimated without DNA-based data. However, demonstrating the feasibility of this approach to all Parties for their national reporting, requires addressing methodological challenges of using empirical data gathered from diverse sources, across diverse taxonomic groups and for countries of varying socio-economic status and biodiversity levels. Here, we assess the genetic indicators for 919 taxa, representing 5,271 populations across nine countries, including megadiverse and developing economies. Data were available to calculate indicators for each country and taxonomic group (765 taxa [83%] had data for at least one indicator). Additionally, 41% of taxa (n=518) have lost at least one-tenth of their populations (complementary indicator [populations maintained] value < 0.9), while 58% of taxa (n=568) have all populations too small to sustain genetic diversity (headline indicator [Ne 500] value = 0). By comparing taxon indicator values to their GlobalRed List status, range size, and other factors, we found the loss of genetic diversity shown by these indicators would go unnoticed by other biodiversity assessments, highlighting the critical importance of monitoring and conserving genetic diversity using these indicators.

Keywords: biodiversity indicators, COP15, Convention on Biological Diversity, effective population size, populations maintained
Introduction

In December 2022, the United Nations Convention of Biological Diversity (CBD) Kunming-Montreal Global Biodiversity Framework (GBF) was adopted by the 196 Parties. The GBF sets the pathway to achieve the vision of a world living in harmony with nature by 2050, with significant progress by 2030 (CBD, 2022). The conservation of genetic diversity in the GBF is significantly and categorically different from previous commitments (Carroll et al., 2023), and is the first to aim for conserving genetic diversity of all species, not just economically valuable or domesticated species. Until now, genetic diversity of non-economically-important species was neglected from previous CBD strategies and other national and global conservation policies (Hoban et al., 2020; Laikre et al., 2010; Laikre 2010). This was largely due to the complexity and expense associated with genetic information, communication barriers and lack of indicators to track genetic change to inform policy (Hoban, da Silva, Hughes, et al., 2023), 2023; Laikre et al., 2020; Taylor et al., 2017; Vernesi et al., 2008). To address this gap, three genetic indicators were developed to monitor different aspects of genetic diversity, namely (i) loss of genetically distinct populations, (ii) populations being too small to retain genetic diversity, (iii) and number of species with genetic monitoring programs (Hoban et al., 2020). The first two are based on processes leading to loss of genetic diversity, and therefore can be estimated using non-genetic data (Hoban et al., 2020, 2021; Hoban, da Silva, Hughes, et al., 2023; Laikre et al., 2020). These two indicators were adopted in the GBF (Annex 1 of CBD/COP/DEC/15/5), which means that parties will be using these indicators to report on their progress over the next decade.

The genetic diversity indicators were developed using SMART (specific, measurable, achievable, realistic, and timely) criteria (see table 2 in Hoban et al., 2021), and were designed to be relevant to Goal A (“The genetic diversity within populations of wild and domesticated species, is maintained, safeguarding their adaptive potential”) and Target 4 (“to maintain and restore the genetic diversity within and between populations of native, wild and domesticated species to maintain their adaptive potential, including through in situ and ex situ conservation and sustainable management practices,”…) of the GBF (CBD/COP/DEC/15/4). The indicator that measures if genetic diversity between populations is maintained was adopted as a complementary indicator. It focuses on the loss of genetically distinct populations, and it is estimated as the number of populations that currently exist over the number of populations that originally existed, i.e. the proportion of maintained populations within species (PM indicator hereafter). To estimate this indicator, it is necessary to spatially define and count populations, which is perceived as one of the scientific challenges to estimate the indicators (Hoban, da Silva, Hughes, et al., 2023)).

The indicator that measures if genetic diversity is maintained within populations was adopted as headline indicator A.4 (mandatory for countries to report). It focuses on populations being large enough to retain genetic diversity, and it is estimated as the proportion of populations within species with an effective population size (Ne) greater than 500 (Ne 500 indicator hereafter). It leverages established theory and empirical data on population genetics stipulating that when populations are below approximately Ne 500, loss of genetic diversity accelerates due to increasing genetic drift intensity (Gilpin & Soulé, 1986; Jamieson & Allendorf, 2012). Importantly, in the absence of genetic data the Ne of a population can be
approximated using the census population size of mature individuals (Nc) and a ratio
between Ne and Nc. The Nc:Ne ratio varies depending on the species breeding strategy, sex
ratio and variance on reproductive success (Frankham, 1995; Waples, 2002), so it can be
adjusted by taxonomic group or even by population. If the ratio is unknown, a conservative
ratio of 0.1 (i.e. Ne being equivalent to 10% of Nc) can be used (Frankham, 2021; Frankham
et al., 2017; Hoban, Paz-Vinas, et al., 2021; Palstra & Ruzzante, 2008).

Other processes that can affect genetic diversity, such as undesired gene flow with
introduced populations or genetically modified organisms, inbreeding, or selection bias
caused by human activities, do require genetic data to be monitored (O’Brien et al., 2022).
For these situations a third indicator was proposed (Hoban et al., 2020; Laikre et al., 2020),
which is the number of species in which genetic diversity has been or is being monitored
using DNA-based methods (genetic monitoring indicator hereafter). This indicator is not
included in the GBF, but countries can report on it, if desired.

To assist the CBD and other stakeholders in compiling relevant data and quantifying these
indicators, we recently developed a standardized, reproducible, and flexible workflow, with
freely accessible guidelines and tools for estimating them (refer to Supplementary Material in
Hoban, da Silva, Mastretta-Yanes et al., (2023) and examples for estimating these indicators
for certain taxa (Hoban, da Silva, Hughes, et al., 2023; Thurfjell et al., 2022). However,
concerns remained over the feasibility of reporting on these indicators for a large number of
species, especially for biologically rich, developing economy nations where financial
resources for biodiversity conservation and monitoring are generally more limited and where
biological data (genetic or non-genetic) are perceived to be less readily available.
Furthermore, some methodological concerns remained, including how to define populations,
assessing population extinction, and ability to use different sources of data to estimate the
indicators (Hoban, da Silva, Hughes, et al., 2023)

In this study, we aimed to address these concerns through a multinational application of the
workflow described in Hoban, da Silva, Mastretta-Yanes, et al. (2023) so as to conduct the
first global assessment of genetic diversity status, with emphasis on the PM and Ne 500
indicators. Nine countries, across six continents ranging in economic status and biodiversity
richness were included: Australia, Belgium, Colombia, France, Japan, Mexico, South Africa,
Sweden, and the United States of America. Five of these countries are megadiverse in terms
of biological richness (Australia, Colombia, Mexico, South Africa, and USA; Mittermeier et
al., 2005); three are developing economy countries (Colombia, Mexico, South Africa:
WorldData.info). Within each country, researchers or practitioners from a range of institutes
including government and non-governmental organisations undertook the assessments. Our
specific objectives were to (1) evaluate data availability across countries, taxonomic groups
and indicators; (2) evaluate whether methods for defining populations influence indicator
values; (3) quantify the distribution of indicator values across taxonomic groups and
conservation status; and (4) to provide guidance and possible solutions to facilitate the
calculation and uptake of the genetic diversity indicators at a global scale.
Results and Discussion

Data are available and it is feasible to report the genetic diversity indicators

We aimed to assess a minimum of 50 species (ideally 100), subspecies or similar (hereafter referred to as taxa) per country. Discretion was given in the specific approach used for selecting taxa and compiling the relevant information in each country, as this would better reflect how each Party would make their own decisions and have different sources of data. However, for each country, we aimed to represent different taxonomic groups within animals, plants and fungi, from among terrestrial and aquatic ecosystems and of varying range size (i.e., range-restricted or wide-ranging, with different levels of extinction risk status and varied life history traits.

A total of 982 assessments were submitted representing 919 taxa, with 50 to 160 taxa assessed per country (Fig. S1a). The total number of populations varied from 5,271 to 5,652, because a subset of 44 taxa were assessed more than once within a single country (Fig. S1b). These multi-assessments allowed us to account for uncertainty in defining populations or their size (Fig. S2). Showing uncertainty is advantageous, as it more accurately reflects knowledge, allows the use of more data, and can highlight gaps and priorities for data collection (Hoban, da Silva, Hughes, et al., 2023). The indicator values of multi-assessed taxa were averaged and counted as a single record in subsequent analyses.

Time to complete a single assessment averaged 3 (sd=1.7) hrs for most taxa, ranging from 2 (sd=1.4) hrs for taxa where information was readily available and where populations were well defined geographically; to 5.5 (sd=3) hrs for more difficult taxa. To put these numbers into perspective, a recent review of genetic studies measuring Ne (Clarke et al., 2023), found that 712 papers published during the past decades estimated Ne in around 3,500 populations, whilst we managed to evaluate more than 5,000 populations in less than a year.

The assessments were distributed across a variety of taxonomic groups for each country; however, assessors from Japan and Colombia predominantly focused on a single taxonomic group (plants and birds, respectively Fig. 1a), to examine if their agencies could leverage on-going monitoring projects, most-updated systematized data and informatics’ pipelines to estimate the genetic diversity indicators. Overall, 83% (765 out of 919) of assessments had data available to report on at least one of the two indicators. For the PM indicator, 57% (565 out of 982) of assessments had data on the number of extinct populations, but countries differed considerably in their confidence to state that a population has been lost (Fig. 1b). For example, assessments from Sweden and Japan considered all taxa without information on extinct populations as having maintained all their populations (i.e., there is no missing information). Therefore, for the PM indicator, in-country decisions drive the distribution of missing data (Fig. S3), while the distribution of missing data is heterogeneously distributed across taxonomic groups and methods to define populations (Fig. S4).
Figure 1. Taxa assessed and data availability by country (including multi-assessments). (a) Heat map showing the number of species or subspecies (taxa) assessed for a given taxonomic group within each country, counting multi-assessed species only once. (b) Total number of taxa with data on the number of extinct populations, as needed for the PM indicator. (c) Proportion of populations within each country with data on population size, as needed for the Ne > 500 indicator. Nc ratio (point) represents more precise estimates of population census size, such as count estimates or approximate values (e.g. capture-recapture study found 3,120 individuals). "Nc ratio (range)" are more generic estimates of census size either represented by quantitative ranges or qualitative descriptions of population size (e.g., "a few hundreds", ">5000 by much", see details in Methods and Materials). "NA" represents populations lacking size data. Within each indicator, all taxa with missing data were removed from subsequent analyses. (Note: pteridophyte includes ferns and similar plants, and bryophyte includes mosses and similar plants).

For the Ne 500 indicator, 64% (614 out of 982) assessments had population size data for at least one population and 14% (131 out of 982) had data at the taxon level (Fig. S5) either in the form of Nc as a point (count) estimate or as a broad range (e.g. “1000-2000”, “< 5000 by much”; see Materials and Methods), or Ne estimated from genetic data (Fig. 1c). Population size data were more commonly available for some taxonomic groups than others. Typically,
angiosperms, mammals and birds had more population level data available as compared to e.g. invertebrates (Fig. S5). Census data made up the vast majority of population size information used to quantify Ne (22% and 53% for point and broad range estimates, respectively), so that Ne was estimated from genetic data in only 6% of the populations in any of the nine countries (Fig. 1b). Allowing for Nc data to be provided in a broad range allows local knowledge holders, including indigenous peoples and local communities, to contribute to the monitoring. Their population size estimates may not be quantitative enough for conventional ecological and evolutionary models, but they are sufficient to qualify if a population is above or below Ne 500. Given that these estimations are conducted at the local scale (e.g. individual populations that local people know well), they may be more robust than similar population size estimations encompassing the entire species range, which typically entail greater inherent uncertainty and assumptions (Jędrzejewski et al., 2018; Wilson et al., 2011). A focus on populations highlighting the importance of local information can also provide empowerment and pride, thereby strengthening community-based conservation efforts (Hoban, da Silva, Hughes, et al., 2023).

The genetic monitoring indicator (number of taxa for which genetic monitoring based on DNA methods is on-going) was also reported by most of the countries, with 6 to 20 species currently being monitored by country (Fig. 5b).

A variety of data are acceptable for defining populations

Populations can be defined using a variety of methods depending on biology and data availability (Hoban, da Silva, Hughes, et al., 2023). We asked assessors to record the approach used for defining populations into the following categories or their combinations: “genetic clusters”, if genetic data defining population structure was available; in the absence of genetic data, populations were delineated using “geographic boundaries” (e.g. different islands), “eco-biogeographic proxies” (e.g. different life zones or biogeographic regions), “management units” (e.g. the unit at which an agency manages), clustering occurrence points within areas within the known dispersal distance of the species (“dispersal buffer”), or “other”. Considering the varied methods employed by each country to define populations (Fig. S6) and the expectation that wide-ranging and restricted species would behave differently, we assessed the impact of method and range type on population counts and indicator values, considering associations between these variables and controlling for variation among countries (Tables S1-S15).

Wide-ranging species were found to have significantly more populations than range-restricted species, when controlling for the method used to define populations (Fig. 2a, Fig. S7, Table S1). Namely, for historically wide-ranging species, defining populations by genetic clusters tends to identify fewer populations encompassing larger geographical areas, than other methods. With respect to indicator values, the “genetic clusters” method (either alone or in combination with other methods) was associated with statistically significantly higher values of both the PM (p = 0.039, Fig. 3, Table S4) and Ne 500 indicators (p = 0.028, Fig. 3, SI Table S10). We found that when controlling for species range, method was no longer a statistically significant predictor of the PM indicator (Table S9). However, using genetic clusters to define populations was associated with higher values of the Ne 500
indicator than when other methods were used, even when controlling for species range (Table S15). This reflects differences between species distribution types: populations covering larger geographic areas may contain more individuals compared to smaller areas. No other consistent relationships between methods and indicator values were found.

Figure 2. Aggregated results across all nine countries examining the associations between the method used to define populations, the number of populations maintained for any given taxa, and the indicator values for the proportion of populations maintained within a taxa. (a) Boxplot showing the spread in the number of populations for each method applied; (b-c) Boxplots showing the range in indicator values across each of the methods applied, for the PM and Ne > 500 indicator, respectively; and (d-e) Violin plots showing the range in indicator values across species range types, for the PM and Ne > 500 indicator, respectively. In all plots, each dot represents the indicator value for a single assessment. Red dots highlight taxa where genetic methods, alone or in combination with others, were used to define populations. n, sample size, is shown to the left of each plot. Outliers with more than 500 populations were removed from these plots and statistical analyses.

Our findings show that a variety of methods can be used to define populations. We acknowledge that genetic markers may not always reveal population boundaries, a limitation which also applies to any method (e.g. use of ecoregions, etc). A potential solution to improve the representation of genetic diversity in widely-distributed species, is to account for uncertainty by defining populations with different methods, including for instance occurrence over different life zones or regions where differentiation would be expected (e.g. Khoury et al., 2019; Tobón-Niedfeldt et al., 2022), and subsequently calculating averages or displaying confidence intervals. For use of indicators in practice, countries will need to document the chosen method transparently and reproducibly, so that the same approach can be applied when re-evaluating the taxa over time.

The genetic diversity indicators reveal loss of diversity otherwise unnoticed
We found that 41% of taxa where we could estimate the PM indicator (n=518) have lost at least 1 out of every 10 of their populations (PM indicator <0.9), and 3% have lost 3 out of 4 or more populations (PM indicator <0.25; Fig. 3b). With each extinct population, private genetic diversity may have disappeared, so even if the species is re-introduced to the area at a later stage, the full genetic diversity of the species would not be recovered (at least not quickly). Loss of populations also changes the ecosystem biotic interactions, which has profound cascading consequences ranging from co-extinctions to the loss of ecosystem services (Young et al., 2016). Early estimates suggested that population loss in tropical forests could occur 3–8 orders of magnitude more rapidly than species loss (Hughes et al., 1997), and yet this loss of diversity is seldom reported. The PM indicator allows us to track these losses of diversity and, with the right actions, prevent it. This is less extreme than the range losses reported by Ceballos et al. s’ (2017) study, which found that all 177 mammals examined had lost at least 30% of their range size, though the authors acknowledge that most of their species were medium to large-sized mammals. Our results are encouraging in this regard, because we found that 54% of taxa still maintain all their populations (PM indicator = 1; Fig. 3b), which also means that a large part of the genetic variation within taxa is still maintained, for now.

Although the findings of the PM indicator are encouraging for some taxa, the Ne 500 indicator shows that the vast majority of populations are below a threshold for maintaining genetic diversity. In 58% of taxa where we could estimate the Ne indicator (n=568), all populations are below the threshold (Ne 500 indicator = 0; Fig. 3d), while only 19% of taxa have all populations above (Ne 500 indicator = 1; Fig. 3d), and overall 87% of all 5,652 assessed populations were below the Ne 500 threshold. We note that Ne 500 does not signal an immediate drastic decline of a species’ genetic health, but it is the point at which genetic erosion starts to accelerate. The effects of inbreeding start at even lower Ne (often Ne <50; Franklin, 1980) and even then, some populations may have evolved (e.g. by purging deleterious alleles) to tolerate small population sizes, for example dwarf island foxes (Robinson et al., 2018) or Ethiopian wolves (Mooney et al., 2023). However, the indicator is set at a threshold of Ne 500 because around that value populations start to lose genetic diversity due to genetic drift (Crow & Kimura, 1970; Frankham et al., 2014; Jamieson & Allendorf, 2012; Willi et al., 2022). Diminishing genetic diversity subsequently lowers populations’ ability to adapt to changing conditions.

Similar trends were found for all taxonomic groups (Fig. 3ab), although in our dataset invertebrates and angiosperms showed smaller values of the PM indicator (higher population loss). Importantly, even in wide ranging taxa, the Ne 500 indicator is skewed towards lower values (Fig. 2e). This is worrisome, because wide-ranging species tend to be considered of less conservation concern. In line with this observation, the values of both indicators are heterogeneously distributed across IUCN Red List status, with low values of the indicators occurring in even Least Concern and Near Threatened species, which would not be considered threatened (Fig. 4). Since IUCN Red List assessments are conducted on a global scale, while our assessments were performed at the national level, often within subsets of species ranges, our results and the Red List are not directly comparable. To investigate the potential impact of calculating Ne at the species level rather than the population level, we aggregated all the Ne values for each taxon and assessed whether the resulting indicator
value exceeded 500 (Fig. S9). Interestingly, 63% of taxa with an aggregated Ne above 500 (black dots in Fig. S9) have an Ne 500 indicator <0.90, and in 17% taxa none of their populations were large enough (Ne 500 =0). In other words, the Ne 500 indicator detects that populations are decreasing in size and losing diversity, which goes unnoticed by species-level assessments.

The success (or failure) of species conservation depends on local decisions affecting each population. The genetic diversity indicators highlight the loss of diversity at the population level, which otherwise goes unnoticed. Thus, the indicators are not only useful to report genetic diversity conservation, but to direct action and policy towards those populations, species or even geographic regions, most are in need.

**Figure 3.** Aggregated results across all nine countries showing the indicator values across taxonomic groups. The spread in indicator values is shown in the violin plots for the (a) PM indicator and (c) Ne > 500 indicator across taxonomic groups; as well as the frequency barplots, grouped according to Kingdom (b, d). In a) and b), each dot represents the indicator value for a single assessment, with the sample size, n, for each taxonomic group provided.
Figure 4. Violin plots illustrating the spread in (a) PM and (b) Ne > 500 indicator values across IUCN Red List categories. Species were classified by their Global Red List status. Abbreviations reflect official IUCN Red List categories, with the global classification used. Sample sizes, n, are provided for each threat category.

Towards addressing genetic diversity conservation at a global scale

The results of this first global assessment of genetic diversity using the GBF indicators shows it is feasible and affordable to estimate them, for middle income and megadiverse countries, as well as a wide range of taxonomic groups, and using data that is already available. Our dataset does not represent the formal assessment of the indicators within the participating countries, but the collaborative experiences obtained throughout this project provide valuable insights, which will be useful for integrating the genetic diversity indicators into National Reports and National Biodiversity Strategy and Action Plans at the global level.

Some common questions parties have is what kinds of species should be included to assess the indicators at the country level, how to report on the indicators and how long the assessments take. Here, we have shown that it is feasible to assess more than 100 species per country that reflect diverse ecosystems, taxonomic groups, range types and life history
traits. These species could be a subset of other lists that countries already have for monitoring or conservation priorities settings. Parties can then summarise the indicators at the national level, or separately by taxonomic group or ecosystem type (Fig. 5). We have also shown that assessing non-threatened species is critical, because the genetic diversity indicators help to reveal diversity loss that might otherwise go unnoticed.

![Table](image)

**Figure 5. Example values for the three genetic diversity indicators.** a) Extreme values for the proportion of maintained populations within species (PM indicator) and the proportion of populations within species with an effective population size (Ne) greater than 500 (Ne 500 indicator). b) Example national indicator values for three genetic diversity indicators. Mean indicator values ± standard deviations provided for PM and Ne 500 indicators. Sample sizes used to quantify these two indicators provided in brackets. Mean values were calculated across all available species (simple equation [Equation 1] in Hoban et al. 2023). Note: The values provided here are examples and should not be considered the official genetic indicator values for CBD national reporting. Countries may choose to adopt a specific priority list of species and additional methods with which to calculate their official values for CBD national reporting. Genetic monitoring, number of species being monitored using DNA-based methods c) Indicator values for South Africa broken down by taxonomic group. Mean values were calculated across all available species within a given group (simple equation [Equation 1] in Hoban et al. 2023). Total indicator values were calculated by averaging all groups together (see Equation 3 in Hoban et al. 2023). This latter equation accounts for possible unequal representation of groups by summing the average indicator values for each and then taking their average.

Importantly, although the PM indicator was adopted as complementary (non-mandatory), we encourage Parties to report it jointly with the Ne 500 indicator. To ensure that Goal A and Target 4 are fully met - maintaining species’ adaptive potential and reducing extinction risk, the most ideal situation would be to have both PM and Ne 500 indicators reported together, but if the Ne 500 indicator is used alone, it must be adjusted to incorporate local population loss (see detailed discussion in Hoban, da Silva, Hughes, et al., 2023). Note that, pragmatically, the Ne 500 indicator is estimated at the population level, which implicitly involves having some knowledge about populations’ delimitations. This means that the effort to estimate the PM indicator when data is available for estimating the Ne 500 would involve minimal additional work.
As for the time it takes, we estimate that 100 species could be assessed in around 300-400 hrs, with around 3 hrs per species. This is already orders of magnitude faster than what it takes to perform conventional genetic studies. However, if coordinating with other processes, this time could be reduced even more. For instance, of the 136 countries who submitted the 6th CBD national report, 61 have a national Red List for at least one taxonomic group and 62 other nations are currently in the process of establishing one (Raimondo et al., 2023). If the data and experts are already gathered for the Red List workshops, we estimate that assessing the genetic diversity indicators should only take 10-20 mins per species, provided that the relevant data, similar to the data we employed in this study, is accessible. Further development of the type of tools we have used, linking them with existing biodiversity databases, species spatial predictions of density and distribution (Jędrzejewski et al., 2018) and earth observation data (Schuman et al., 2023) could also help to estimate the indicators for hundreds of species in a semi-automatic way, involving human intervention but minimizing labor.

Capacity building needs will depend on whether the countries: (a) already have available data (on population sizes, for example, or occurrence points in geographic information systems) in a centralized database, with little to no knowledge gaps, (b) do not have a centralized database but with some available data in various resources and small to medium science gaps, or (c) have little or no data, with significant data gaps. For this, “available data” should be considered broadly, including citizen science, grey literature, local experts’ knowledge, and informal data held by small NGOs and local communities, and not only scientific data coming from ecological or genetic studies.

The fact that genetic studies are not needed to estimate the indicators does not mean that genetic data is not desired; on the contrary, genetic studies will remain an important source of information for more accurate estimations of the indicators, and for punctual management actions and genetic monitoring of some species. The PM and Ne 500 indicators can help countries to decide which species, or populations, need genetic studies, either because census data shows they are too small and hence genetic studies are needed to guide interventions (for example by breeding or informing translocations), or because they could be affected by other processes affecting genetic diversity not covered by the PM and Ne 500 indicators. For example, the Mexican species with genetic diversity monitoring programs focus on crop wild relatives, where gene flow with genetically modified organisms and improved varieties are a concern (Rojas-Barrera et al., 2019; Wegier et al., 2011); in the Swedish assessments, cod, salmon, and moose (Dussex et al., 2023; Johannesson & Laikre, 2023) are subject to heavy harvest and are monitored with DNA methods within the framework of Sweden’s recently initiated national program for monitoring genetic diversity with DNA techniques (Andersson et al., 2022; Johannesson & Laikre, 2020). Thus, although the genetic monitoring indicator was not adopted at GBF, countries are already undertaking DNA-based monitoring schemes in some species, and many more already have genetic data, even if it was not generated for monitoring purposes (Fig. S10).

The genetic diversity indicators also open up interesting new avenues for research, including the effects of past demographic history on how species cope with small current Ne, as well as the genetic basis of how populations adapt to changing conditions. Meanwhile, in the
context of growing environmental changes in the Anthropocene, the present assessment highlights a concerning trend—a decline in genetic diversity. Yet, this is also a critical opportunity: to safeguard and enhance genetic diversity precisely when species need it the most.

**Materials and Methods**

During late 2022 we (members of universities, research or governmental institutions of the nine countries listed above) co-developed guidelines, metadata and a web-form to collect data on the genetic diversity indicators in a reliable, standardized and reproducible way (Hoban, da Silva, Mastretta-Yanes, 2023). This resulted in the production of a project guidance document to help harmonize the intentions of the project, as well as convey the principles in quantifying the indicator; and a standardized set of questions encompassing the data to estimate the indicators (number of extant and extinct populations, and the population size of each one), and metadata on the species (including taxonomic group, life history traits, extinction risk status, distribution type, among others), and sources of information (references, names of people providing information). The questionnaire was converted into a Kobo form using KoboToolBox (https://www.kobotoolbox.org/; a free and open source tool for data collection and management), which was then used for participants of the project of data collection. This allowed us to standardize data collection and prevent common errors in data-capture. Once all assessments were completed, the dataset was downloaded as a .csv file and processed in R version 4.2.1 for data-quality check, indicators estimation (see below), analyses and plotting. For this a series of custom functions and processing pipeline was developed (available at github.com/AliciaMstt/GeneticIndicators). To examine the associations between method to define populations, species range type, country of assessment with number of populations and the indicator values, we used generalized linear mixed models (glmer) and generalized linear mixed models via template model builder (glmmTMB). The indicator values of multiple assessments of the same taxon within a country were averaged and used as a single entry for the statistical analyses and indicators plots.

**Species selection**

Each country selected approximately 100 species to assess, and then gathered all relevant information to aid in the quantification of the three genetic indicators. While all countries followed the same principles and answered the same questions, discretion was given in the specific approach used in selecting taxa and compiling the relevant information. Details in Supplementary Text S1.

**Defining populations**

A checklist of five different methods typically used to define populations, plus an option to include additional approaches, was presented to participants as one of the questions in their species assessments. These methods were: Genetic clusters/clades, Geographic boundaries, Ecological or Biogeographic proxies, Traits (e.g., behavioural, morphological, physiological), Management Units (demography/ migration) and Dispersal Buffers. Participants could select all methods that applied based on the available information
compiled for a species. Additional free-text questions required assessors to briefly explain how populations were defined providing a short narrative and references.

*Estimating the PM indicator*

Given that we are interested in human-induced changes in species’ genetic diversity and structure, a baseline time period of 50–200 years ago was suggested to help quantify the number of extinct populations - a timeframe reflecting the industrial era when rapid habitat changes occurred in many countries. However, considering baselines could vary by country depending on human impact and growth, and may depend on the species and what data are available, participants could use discretion in setting a baseline time period for assessing extinctions. The year or period of years used as baseline was recorded in a species-case.

For species where populations could be defined, the proportion of the number of existing populations (currently present; extant) against the total number of known populations (sum of extant and extinct) was determined (i.e. PM indicator). For species where the number of extinct populations was classified as unknown, the PM indicator was not calculated.

*Estimating the Ne> 500 indicator*

For species with census or effective population size data for at least one population, the Ne 500 indicator was calculated as a proportion of the number of populations with Ne > 500 against the total number of extant populations for a species. Only populations with Ne or Nc data were considered in calculating the indicator; thereby reducing the number of total populations in the denominator.

For Nc data provided as a semi-qualitative measure or as a range, generalized Nc values were assigned to facilitate computation of Ne and hence the Ne 500 indicator. As a default, when Ne data was lacking, yet Nc data was available, a Ne:Nc conversion ratio of 0.1 was applied to roughly estimate contemporary Ne from Nc.

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