Chapter 1
A Fresh Look at Conservation Genetics in the Neotropics

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1.1 Introduction

It has been more than a century since genetics was first used to interpret an ecological response, when a mutant gene was reported to have been selectively eliminated by predation in caterpillars (Gerould 1921), thus founding what would later become known as ecological genetics. This discipline is defined by the study of the genetic bases of an organism's adaptation, i.e., the adaptations of wild populations to their environment (Ford 1964). Since then, genetics, evolution, ecology and conservation have been continuously intertwined, motivating many researchers to think about new challenges and propose new disciplines. Thus, the last ten decades have been a period of great transformation of our knowledge about all kinds of living organisms and their persistence on Earth. However, human activities have led to severe changes on our planet, resulting in a rapid loss of species and populations, and characterizing a true global biodiversity crisis (Bellard et al. 2012; Dirzo et al. 2014; Haddad et al. 2015). Many efforts have been made to mitigate the impacts of human activities on biodiversity, as it is a well-established fact that genetic diversity plays a crucial role in the long-term persistence of species and populations (Hoban et al. 2023). Therefore, the use of genetics can provide important information and emerge as a powerful tool for conservation and effective decision-making (Torres-Florez et al. 2018).

In this scenario, conservation genetics emerged as an application of genetics in the scope of biological conservation, which later became a multidisciplinary field of study marked by the perception that the disorderly growth of human activities has a huge impact and jeopardizes local and global biodiversity. The birth of conservation

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genetics dates back to the early 1970s, with the papers of Sir Otto Frankel (Frankel 1970; Frankel 1974), a plant geneticist who first recognized the genetic importance for conservation. In the emblematic book *Conservation and Evolution*, Frankel and Soulé (1981) presented genetic problems associated with conservation, explored the meaning of genetic diversity for the maintenance of populations and ecosystems, and highlighted principles and practices of conservation genetics for the long-term conservation of nature. This established the foundations of conservation genetics. Later, Frankham et al. (2002) defined conservation genetics as a discipline that uses theoretical and methodological approaches of genetics to save species and populations facing the risk of extinction. A central idea in conservation genetics is that small, isolated populations can be threatened by the occurrence of random genetic drift and inbreeding (Ouborg et al. 2010). Genetic drift, defined as the random fluctuation of allele frequencies across generations, can lead to the random loss or fixation of alleles when it occurs in small populations. This can result in the loss of adaptive alleles, or in the fixation of deleterious alleles. Inbreeding, on the other hand, increases the frequency of homozygotes, which can expose deleterious alleles and lead to inbreeding depression, i.e., a reduction in individual fitness on average. Although both genetic drift and inbreeding can occur in large populations, their effects are much more pronounced in small populations, and at least three important consequences can result from these effects. In small, inbred populations, the reduction of individual fitness can decrease their viability in the short term. In addition, the loss of genetic variants in a small population can compromise its evolutionary adaptive potential, and reduce its long-term viability in a changing environment. Finally, genetic drift, independently occurring in small and isolated populations, may promote genetic divergence between them, compromising the genetic unity of the species. This may also lead to outbreeding depression, if the gene flow between the groups is restored (Frankham et al. 2017; Frankham et al. 2002).

The increasing development of molecular technologies has allowed researchers to evaluate these theoretical effects on small populations and confirm conservation genetics as a well-established, empirical discipline. However, despite the great expansion of conservation genetics worldwide, it remains disproportionately understudied in the Neotropics. The Neotropics harbor a huge biodiversity, with endemic species which have been increasingly threatened by habitat degradation and wild population decline, which could lead to high rates of extinction in the future (Dirzo et al. 2014). Despite all this, between 1992 and 2014, publications on conservation genetics from Latin America featured almost ten times less frequently in indexed journals than those from European countries (Torres-Florez et al. 2018), and there is no reason to believe that this scenario has changed in recent years. Many factors may be causing this disproportionality, but it is clear that conservation genetics in the Neotropics has much to contribute to the conservation of this important biodiversity.

1.2 Conservation Genetics at the Species Level

Traditionally, conservation genetics has been focused on biological diversity at the species level, and, while its main goal is to save endangered species from the risk of extinction (Frankham et al. 2002), many studies published worldwide in the field of genetics applied to conservation have been targeting on non-threatened species (Torres-Florez et al. 2018). Genetics – applied to a species or its populations – has been utilized for a range of general topics in biodiversity conservation, from resolving taxonomic uncertainties to the long-term monitoring of populations. Genetics has been employed around the world to assist in various aspects of conservation, such as defining evolutionary significant units (ESUs) and/or management units, minimizing inbreeding and loss of genetic diversity in populations, managing captive populations for reintroduction, assessing invasive species and their impacts on threatened species, estimating sex ratio, population size and demographic history, contributing to management plans and forensic actions, and predicting extinction risk and responses to environmental changes (Frankham et al. 2017; Torres-Florez et al. 2018); more recently, the use of genetics in conservation has been increasing in the Neotropics as well.

Fish, for instance, are among the most diverse groups of neotropical vertebrates, and present several taxonomic challenges. Morphologically similar species often form what is known as a species complex, in which species are virtually indistinguishable through their morphology or color patterns. In these cases, genetic tools, such as DNA barcoding (using the COI gene) or other molecular gene identification techniques, have revealed a significant hidden biodiversity (Pires et al. 2017; Ramírez et al. 2017a; Silva-Santos et al. 2018). Molecular analyses have been integrated with chromosome and morphology studies to describe new species (Garavello et al. 2021) or even entirely new genera (e.g., *Megaleporinus*, Ramírez et al. 2017b).

Indeed, there is no major living taxon that has not received some contribution from genetic investigations for the identification of hidden biodiversity, either by chromosomal analyses, molecular methods, or both. Metagenomics has revealed to science numerous new bacteria and archaebacteria, most of which are known only as Operational Taxonomic Units (OTUs) and have been deposited in public databases such as GenBank, but still require further biological characterization. Plants and animals have also benefited from the power of molecular tools to reveal their hidden biodiversity. Molecular phylogenies and species delimitation methods, as well as DNA barcoding, can be included in a growing body of methodologies which have proven to be useful in revealing hidden biodiversity in plants (Vijayan and Tsou 2010; Lima et al. 2018) and in most animal groups (Ahmed 2022; Fišer and Buzan 2014) all over the world, including the hyperdiverse Neotropics.

The number of genetic population studies that have contributed to the conservation of threatened species in the Neotropics has increased significantly. However, for various reasons, most studies are conducted on species of low conservation concern. In a world increasingly devastated and fragmented by human actions, reduced gene flow and the loss of genetic variation have been described in several

populations of neotropical plants (Vitorino et al. 2020), fish (Machado et al. 2022), birds (Banhos et al. 2016), and mammals (Ayala-Burbano et al. 2017). Inbreeding in small populations has also been reported in fish (Langen et al. 2011; Coimbra et al. 2020), frogs (Nali et al. 2020), and mammals (Collevatti et al. 2007). It is worth noting that, until the last review in 2014, around 40% of the conservation genetic studies in Latin America had focused on population structure (Torres-Florez et al. 2018), highlighting the concern of researchers in clarifying the genetic conse-quences of habitat fragmentation. There is no reason to believe that this concern will diminish in the near future. While ESUs have been suggested in fish species such as *Pseudoplatystoma corruscans*, which has a wide distribution in various hydro-graphic basins (Carvalho et al. 2012), genetic analyses have recently been used to evaluate the translocation and reintroduction of a small endangered primate (Moraes et al. 2017), *Leontopithecus rosalia*, which was successfully done a few decades ago. Several other genetic studies have focused on *ex situ* populations, which can serve as an insurance policy for an endangered species by producing new individu-als that can help in recovering wild populations at risk of extinction. However, *ex situ* populations are often comprised of a reduced number of individuals, and are prone to showing loss of genetic variation, inbreeding, and inbreeding depression, which have been investigated in primates (Ayala-Burbano et al. 2017, 2020), for instance, or hybridization, which has been investigated in birds (Costa et al. 2017).

Genetics applied to conservation has already made meaningful contributions to the knowledge of species and their populations, and it has shown great potential to help monitor and manage the *in situ* and *ex situ* populations of threatened species. Expanding these studies could lead to significant progress, particularly for the per-sistence of biodiversity in the hyperdiverse Neotropics.

1.3   Genetics for Studying Communities and Ecosystems

Biodiversity has long been recognized as encompassing not only the diversity of species, but also genetic and ecosystem diversity (Wilson 1988). While the primary goal of conservation genetics is to preserve endangered species and their genetic diversity, it is noteworthy how genetic and molecular tools can also aid in the con-servation of communities and ecosystems, such as in identifying priority areas for community conservation. At least three important approaches – phylogenetic diver-sity, comparative phylogeography, and environmental DNA – can illustrate the pow-erful use of genetics and molecular information to contribute for a better understanding of structures and functions of the biological diversity present in diverse ecosystems.

1.3.1 Phylogenetic Diversity

Phylogenetic diversity (PD) was introduced by Faith (1992) to address the goal of conserving biodiversity at the environmental or community levels, rather than just assessing biodiversity at the species level. There are various metrics for estimating phylogenetic diversity, all of which are most frequently based on molecular data, making genetic information essential for these studies. The most commonly used metric, Faith's phylogenetic diversity (PD_{Faith}) (Faith 1992), is based on cladistic information, i.e., it is the sum of the lengths of all branches in a molecular phylogenetic tree containing a set of taxa from the entire community. PD is strongly correlated with species richness (Tucker et al. 2017), whereas other metrics based on the average pairwise genetic distance of all species (MPD, Webb 2000), or on the average pairwise genetic distance between closely related species (MNTD, Webb et al. 2002), are less dependent on species richness. Higher MPD (Mean Pairwise Distance) values indicate that the assessed set of species in the community are from a wide range of clades, whereas a high MNTD (Mean Nearest Taxon Distance) suggests that closely related species do not co-occur in the community. Regardless of the metric used, a more complex and less redundant local community will show higher phylogenetic diversity, suggesting higher priority for the conservation of a broader biodiversity. In contrast, a local community showing lower phylogenetic diversity might indicate a local loss of species, and might be a measure of the impact of habitat loss.

Phylogenetic diversity has been assessed in several taxa in the Neotropics, such as in plants (Perea et al. 2022), bees (Antonini et al. 2017), birds (Hanz et al. 2019), and mammals (Gómez-Ortiz et al. 2017), but few studies have primarily focused on conservation. For example, PD was used to evaluate the impacts of habitat loss on the evolutionary diversity of snakes (Fenker et al. 2014), the effects of oil palm management on bird communities (Prescott et al. 2016), spatial variation in communities of Atlantic Forest opiliones (Nogueira et al. 2019), loss of phylogenetic diversity of bats across a habitat gradient in the Amazon (Aninta et al. 2019), the identification of areas of high mammalian phylogenetic diversity in order to suggest priority areas for conservation (Aguillar-Tomasini et al. 2021), and to guide the conservation of crop wild relatives (González-Orozco et al. 2021).

Indeed, by assessing biological diversity at the community level (Faith 1992), phylogenetic diversity can provide a good picture of the evolutionary history of communities, and how they might respond temporally and spatially to a range of stressors, such as habitat loss and fragmentation. Phylogenetic diversity can also be used to guide large-scale conservation approaches, particularly for protecting the megadiversity of the Neotropics.

1.3.2 Comparative Phylogeography

Phylogeography can be a powerful tool for conservation. This field of study, which aims at understanding the geographic arrangement of genotypes, was first proposed by Avise et al. (1987), and was rapidly recognized as an important approach for inferring population evolutionary history. Phylogeography focuses heavily on describing population relationships within a single species, and has been widely used to study almost all living groups. In plants and animals, phylogeography has revealed several cases of spatial genetic differentiation among populations, potentially contributing to the conservation of these populations. More information on this topic can be found in Chaps. 6 and 21.

With the expansion of these studies, comparative phylogeography (CP) emerged with the aim of understanding the evolutionary and biogeographical history of species that are co-distributed in space. In an integrative work, comparative phylogeography among resident vertebrates in the wet tropical rainforests identified genetically divergent areas important for conservation (Moritz and Faith 1998). The authors concluded that combining comparative phylogeography (population level) with phylogenetic diversity (species level) could improve biodiversity conservation planning. It is impressive to observe how much comparative phylogeography applied to conservation studies has advanced in the few decades since its birth. A quick search on Web of Science, using the terms "comparative phylogenetic*" AND "conservation", revealed almost four hundred papers published from 1997 to 2023. In general, these works combined the phylogeographies of two or more co-distributed species to infer areas of highest priority for conservation. For instance, comparative phylogeography within a crustacean group (*Excirolana*) highlighted the importance of this molecular approach in supporting conservation actions on sandy beaches, an ecosystem highly impacted by anthropogenic stressors (von der Heyden et al. 2020). Similarly, a study involving tree species was used to investigate large-scale conservation corridors in subtropical shrublands, and to support planning decisions for their conservation (Potts et al. 2013).

Comparative phylogeography is still in its infancy in the Neotropics, and has primarily been used to investigate the association between the evolutionary histories of two or more species, and to understand the dynamics of their evolution in different habitats or biomes. For instance, CP has been used in birds and bats to evaluate whether the presence of barriers can promote different phylogeographic patterns among ecologically diverse species (Matos et al. 2016; Loureiro et al. 2020, respectively), to test biogeographic hypotheses in river otters (Ruiz-García et al. 2018), and to investigate the impact of the climate change which occurred in the Pleistocene on orchid bees (López-Uribe et al. 2014). To our knowledge, there are still no comparative phylogenetic studies primarily designed to answer questions on conservation in the Neotropics. Considering the aforementioned potential, the use of CP to identify priority areas in the Neotropics could be valuable for the conservation of its megadiversity.

1.3.3 Environmental DNA and Invertebrate-Derived DNA and Conservation

The use of environmental DNA (eDNA) and invertebrate-derived DNA or ingested DNA (iDNA), in association with modern sequencing technologies, has been increasingly recognized as a powerful tool for biodiversity assessment and conservation (Carvalho et al. 2022; see Chap. 18 for more details). Human-promoted habitat loss and climate change have led to a true global biodiversity crisis (Bellard et al. 2012; Dirzo et al. 2014; Haddad et al. 2015), and a more comprehensive understanding of biodiversity is critical for nature conservation. Traditional methods for surveying species are generally limited to sampling at a local scale and with a substantial effort. New technologies, such as metabarcoding using eDNA and iDNA, can be powerful tools for biodiversity surveys, and for supporting the conservation of natural ecosystems (Carvalho et al. 2022).

Environmental DNA obtained from water, soil, or air can provide more accurate and less time-consuming biodiversity surveys, as it is capable of assessing the species diversity – including rare and elusive species – from a large number of samples and in large-scale surveys, notably reducing labor costs (Bohmann et al. 2014; Rees et al. 2014). In addition, the community of vertebrates can also be assessed through the iDNA obtained from the guts of invertebrates such as flies, mosquitoes, leeches, and beetles (Calvignac-Spencer et al. 2013; Schnell et al. 2015; Kocher et al. 2017a), as easily and efficiently as with eDNA.

According to Taberlet et al. (2012), the term "environmental DNA" first appeared at the beginning of the 2000s, coinciding with the emergence of the earliest metagenomic studies (Rondon et al. 2000; Gillespie et al. 2002). However, the first reference to an eDNA extraction method is credited to Ogram et al. (1987), who described a method for extracting microbial DNA from sediments. Since then, the application of metagenomics to conservation has become a feasible and convenient task. For example, a combination of metagenomics, microscopy, microbe cultivation, and water chemistry, was used to characterize microbial communities in coral atolls, furthering the scientific understanding of the association of microbes with the degradation of coral reef ecosystems across the globe (Dinsdale et al. 2008). Soon after, eDNA began to be used to assess eukaryote communities on a global scale (e.g., Bhadury et al. 2006), mainly through next-generation sequencing and metabarcoding for taxon identification (e.g., Chariton et al. 2010).

Few studies have utilized eDNA or iDNA to evaluate eukaryotic communities in the Neotropics. The first study using eDNA in the Neotropics assessed amphibian communities in Brazilian Atlantic Forest streams, and compared the results with conventional field surveys (Sasso et al. 2017). Of the ten species that had been previously identified – over a five-year period – through visual-acoustic methods, being thus linked with the streams at least during one of their life stages (i.e., egg, tadpole or post-metamorphic), the authors were able to detect nine of them through eDNA metabarcoding from water samples collected over 4 days. This result illustrates how the eDNA method can be beneficial in supporting the conservation of neotropical

amphibians. In the same year, Kocher et al. (2017b) reported short mitochondrial sequences for the identification of Amazon mammals through metabarcoding.

Subsequently, eDNA from water samples and metabarcoding were successfully employed to assess the mammalian communities in two highly biodiverse regions of Brazil, the Amazon and the Atlantic Forest (Sales et al. 2020). To our knowledge, this was the first study aimed at detecting neotropical mammals using DNA extracted from water, an effort in which the potential and challenges of eDNA monitoring for mammals were highlighted. Indeed, eDNA from water bodies has predominantly been used to detect fish communities around the world (for a review, see Carvalho et al. 2022), and this has also been observed in the Neotropics (Cantera et al. 2019; Milan et al. 2020; Sales et al. 2021; Santana et al. 2021; Carvalho and Leal 2023).

On the other hand, iDNA obtained from the guts of insects (either hematophagous, saprophagous, or coprophagous ones) has been predominantly used to assess mammal communities (Calvignac-Spencer et al. 2013; Schnell et al. 2015; Rodgers et al. 2017; Saranholi et al. 2023), although other vertebrates have also been identified (Calvignac-Spencer et al. 2013; Saranholi et al. 2023). iDNA has also been used in ecological investigations, such as dietary studies focused on disease transmission by hematophagous insects (Bitome-Essono et al. 2017), pathogen and virome assessment (Bass et al. 2023), trophic interactions (Paula et al. 2016), and biological control (Paula and Andow 2022). In the Neotropics, the use of iDNA to assess animal communities is still very incipient, and it is mostly dedicated to testing and comparing different insect groups, mainly for surveys focused on mammals (Massey et al. 2022; Saranholi et al. 2023).

An important limitation in the use of eDNA/iDNA and metabarcoding is the availability (or rather, the lack thereof) of reference barcoding sequences, especially when working in the Neotropics. Further efforts are still required to obtain good sets of these sequences. However, due to the relative ease of collecting insects or environmental samples from different biomes, as well as the cost-effectiveness and time-saving benefits of eDNA/iDNA analyses, and their potential for future technological and methodological advancements, these approaches may still become the primary tools for conducting easy and efficient biodiversity surveys worldwide, particularly in the hyperdiverse Neotropics.

1.4   Conservation Genomics in Neotropics

Conservation genomics can be defined similarly to conservation genetics, with the difference being the amount of molecular information available from genomic studies (Avise 2010). Thus, conservation genomics refers to the use of genomic techniques to address problems in conservation biology (Allendorf et al. 2010). The number of genomes sequenced is rapidly increasing and, while the first reported eukaryotic genome sequences were from model species (e.g., *Caenorhabditis elegans*, *Arabidopsis thaliana*, *Drosophila melanogaster*), most of the currently sequenced species are non-model organisms, indicating a growing availability of

genomic information from an ever-expanding number of plants and animals (Ellegren 2014; for more on this, see Chaps. 19, 20, and 21).

Genomic techniques can be categorized into three basic classes (Allendorf et al. 2010). The first one consists of SNP (Single Nucleotide Polymorphism) genotyping microarrays, which are used to detect single-base polymorphisms across the DNA of a population. However, using SNP microarrays or SNP chips suffers from an important limitation. Because an SNP chip is built to be species-specific, its use is often limited to the target species. Thus, considering the high costs associated with their development and construction, SNP microarrays are most commonly designed for either model species or species of great economic interest. For instance, SNP microarrays are used in human genetics, from detecting single-gene mutations (Bruno et al. 2011) to forensic investigations (Voskoboinik et al. 2015). They have also been used for genotyping in animal breeding, such as in alpacas (*Vicugna pacos*) (Calderon et al. 2021). The second class of genomic techniques is reduced-genome representation sequencing – or GBS (Genotyping-by-Sequencing), with methods such as RADseq (Restriction-site Associated DNA sequencing) and ddRADseq (Double-digested Restriction-site Associated DNA sequencing), which employ next-generation sequencing technology to target orthologous regions across the genome of different individuals. For example, ddRADseq has been used to develop a panel of SNPs to investigate population polymorphisms in migratory birds (Larison et al. 2021). A similar approach has been used to investigate diversification within a neotropical toad species, and to test a set of hypotheses concerning reduced gene flow among populations (Thomé et al. 2021). Finally, whole-genome sequencing, which was initially used for describing the genomes of various organisms, from viruses and bacteria to plants and animals, has now been increasingly used in population genomic studies. Public databases (e.g., GenBank) already make a considerable number of genomes available, which have been studied in order to answer a wide variety of questions. It is noteworthy that all these genomic techniques produce vast amounts of data, requiring the critical use of bioinformatics for their analyses (Allendorf et al. 2010).

Population genomics focuses on the variations between genomes and populations (Luikart et al. 2003), and the large-scale resequencing of genomes from various populations could lead to the identification of genes and genomic regions linked to fitness-related traits (Ellegren 2014). Conservation genomics may take advantage of this approach to study the genetic bases of local adaptations, or inbreeding depression (Allendorf et al. 2010). On the other hand, with the use of neutral markers, conservation genomics can also estimate population parameters such as genetic diversity, gene flow, and effective population size, which may be employed to support species management and conservation. Thus, genomic tools have great potential to improve the management of populations for conservation, from estimating the genetic parameters of populations with basis on a large number of neutral markers, to identifying loci linked to local adaptations (Allendorf et al. 2010).

In the Neotropics, genomic tools have been increasingly used for the conservation of plants and animals. For example, landscape genomic analyses have been used to produce insights on the negative consequences of habitat loss, and to

recommend gene flow restoration among populations of endangered turtles (Gallego-García et al. 2019). Fish are among the groups that have been most extensively assessed by genomic approaches in the Neotropics, likely due to the expansion of aquaculture of native species, and to the importance of conserving these resources. Indeed, genomic tools have greatly enhanced our understanding of neotropical fish, and can aid in their conservation. For example, the development of SNP panels for population genetics (Martínez et al. 2016, 2017; Mastrochirico-Filho et al. 2016; Delord et al. 2018), the assessment of genetic diversity in breeding species (Mastrochirico-Filho et al. 2019), and the investigation of hybrid zones in an annual fish genus (García et al. 2019) have provided valuable insights. In addition, the production of linkage maps and the utilization of genome-wide association studies to investigate pathogen resistance (Mastrochirico-Filho et al. 2020; Ariede et al. 2022) and genes linked with the absence of intermuscular bones (Nunes et al. 2020) demonstrate the potential of genomic tools for supporting both aquaculture and conservation efforts.

The Neotropics are primarily composed of low- and middle-income countries that lack the infrastructure to manufacture equipment and chemicals for next-generation sequencing, which has made genomic research in the region difficult due to the high costs involved. Nevertheless, the lowering costs of these technologies have made them more accessible globally, which should have a significant impact on future biodiversity conservation studies in the Neotropics. (For more information, see Chaps. 19, 20, and 21).

1.5   Final Considerations

Conservation genetics is a well-established field of study around the world, including in the hyperdiverse Neotropics. From DNA barcoding to genomics, conservation genetic approaches have been widely used to address a variety of conservation questions in plants and animals, and to offer management actions for target species (Torres-Florez et al. 2018). Still, despite the many achievements of the past decades, several important questions related to selectively important genetic variation, fitness and adaptation, as well as genetic and environmental interactions, continue to challenge conservation geneticists. Most inferences on conservation genetics are obtained from microsatellite-based population studies, but the true value of neutral genetic diversity for species conservation is still under debate (García-Dorado & Caballero 2021; Teixeira & Huber 2021; Hoban et al. 2023). In this context, conservation genomics can offer new opportunities for monitoring changes in allele frequency (both neutral and non-neutral), and for evaluating the effects of genetic drift and natural selection within and between populations (Allendorf et al. 2010), in addition to identifying genes and genomic regions involved in adaptation (Ellegren 2014).

Expanding from the species level to the community or ecosystem levels can help address broader conservation concerns, and using techniques such as phylogenetic

diversity, comparative phylogeography, and eDNA/iDNA can promote new paradigms in conservation genetics. Regardless of the questions being asked, it appears that the importance of conservation genetics and genomics is still poorly understood by decision makers. In their review, Torres-Florez et al. (2018) found few cases of improvements in species protection resulting from published research on conservation genetics. Applying conservation genetics and genomics information to policy and decision making, as well as to the planning and implementation of conservation practices, remains a significant challenge. This is particularly true in the Neotropics, where biodiversity is vast and includes strategic biomes such as the Amazon Forest, which is crucial for global sustainability.

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