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Article

Genetic and Phylogenetic Studies in *Manilkara*: A Review of Molecular Markers

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Abstract

Manilkara (Sapotaceae) includes tropical tree species of high ecological and socio-economic value, yet genetic and phylogenetic evidence remains uneven across taxa and eco-geographic regions. Here, we synthesize studies conducted between 1999 and 2025 which summarize the use of molecular markers to infer genetic diversity, connectivity, population structure, and evolutionary relationships within this genus. The studies are dominated by PCR-based marker systems, including dominant markers (like RAPD and SCoT) and microsatellites from the nuclear genome and plastid genome. Other studies rely on PCR-amplified sequence loci, such as ITS and chloroplast regions, while others use high-throughput technologies, including NGS-assisted SSR development, sequences of complete plastomes, and targeted nuclear sequencing. Overall, studies using SSRs provide the most informative estimates for within-species diversity and fine-scale structure, whereas plastid datasets (cpSSR/cpDNA) mainly support inference on maternal lineages and plastid-based relationships but can be constrained by uniparental inheritance and limited variation, especially under small sampling. Some limitations found include heterogeneous sampling, inconsistency in reporting the methodological parameters, and limited connection with ecological or phenotypic parameters which restricts chances of inferences on demography and adaptation. Based on this review, future research in *Manilkara* would benefit from setting up a broader taxonomic and geographic coverage, incorporating genome-wide technology where feasible to strengthen conservation management, and breeding opportunities in *Manilkara*.

Keywords: genetic diversity; population structure; sapotaceae; technical gaps; genome

1. Introduction

An ecosystem dominated by species of the Sapotaceae family serves as a reliable indicator of forest quality in tropical regions (Gautier, 2003). Sapotaceae grow slowly and produce high quality timber that is increasingly recognized internationally (Christe et al., 2021). Moreover, species of the Sapotaceae family represent key structural elements of Neotropical rainforests, although certain taxa also inhabit seasonally dry environments. The family is characterized by distinctive morphological traits such as the exudation of latex, simple entire leaves, malpighiaceous hairs, and flowers typically grouped in fascicles (De Faria et al., 2017). Because their flowers and fruits are frequently scarce or located high in the forest canopy, they are often difficult to collect or observe (Swenson and Anderberg, 2005; Kümpers et al., 2016). These conditions contribute to the taxonomic complexity of the family. Consequently, Sapotaceae species remain insufficiently investigated from both taxonomic and molecular perspectives across many tropical regions (Christe et al., 2021).

The pantropical plant family Sapotaceae comprises about 1250 species (Govaerts et al., 2001; Pennington, 1991), but it is steadily increasing in tropical America (Pennington, 2006, 2007), Africa (Gautier et al., 2016) and the Pacific region (Swenson et al., 2007; Munzinger and Swenson, 2009; Swenson and Munzinger, 2012, 2016). Currently, this family includes 65–70 genera (Swenson et al., 2020). In Peru, it comprises 10 genera and 90 species, most of which are evergreen trees that produce white latex and have exudations on all parts of the plant (Gentry, 1993). The *Manilkara* genus currently comprises approximately 102 species found in tropical regions (GBIF.org, 2026). Nevertheless, although numerous species face severe extinction risks driven by anthropogenic pressures, information regarding their genetic diversity, population structure, and reproductive biology remains scarce (Pennington, 2008, 1990, 1991).

Tropical forests harbor the highest levels of biodiversity on the planet and include numerous species of the genus *Manilkara*. Nonetheless, these ecosystems have undergone extensive deforestation and degradation as a result of human activities (Dirzo and Raven, 2003; Phillips et al., 2017). The escalating demand for timber, energy, food, and agricultural resources to meet human needs has intensified deforestation, leaving much of the remaining flora and fauna confined to small, isolated forest remnants. This ongoing habitat loss has triggered sharp population declines, accelerating global warming and the consequent erosion of biodiversity worldwide (Laurance et al., 2014; Betts et al., 2019).

The threat extends ranges from the complete disappearance of species to the potential erosion of genetic diversity and the diminished capacity of surviving populations to adapt and recover (Pither et al., 2003; Choi et al., 2024). Deforestation and habitat fragmentation modify landscape structure, biodiversity and natural connectivity (Choi et al., 2024; Taylor et al., 1993). These changes can reduce genetic diversity in multiple ways. (i) Direct loss of genetic variation: when habitats shrink or disappear, their populations and unique genetic traits may be lost due to declining numbers. (ii) Isolation of surviving groups: fragmented populations experience limited gene flow, further reducing genetic exchange (White et al., 2002).

Such processes may lead to genetic bottlenecks, intensify random genetic drift, and increase the likelihood of inbreeding depression (Young et al., 1996; Choi et al., 2024). Reduced seed and pollen dispersal can alter the mating patterns of trees and modify their genetic distribution across landscapes. When habitat fragmentation isolates populations, greater distances between populations increase the likelihood that relatives will interbreed. This increased inbreeding ultimately reduces genetic variation within species (Carvalho et al., 2017).

Molecular characterization of forest species serves as a crucial tool for elucidating their genetic diversity, population structure, and evolutionary relationships, all of which are vital for the conservation and sustainable management of their genetic resources. Different molecular tools exist and have historically been applied according to diverse research objectives. Nuclear markers (SSR, RAPD, SNP) have been typically used to assess intraspecific and population-level diversity, while cpDNA and nuclear ITS regions are commonly used for species identification and phylogenetic inference (Hollingsworth et al., 2011; Avise, 2012). Collectively, these molecular techniques have offered opportunities for investigating, conserving, and managing forest genetic resources. Combining molecular evidence with ecological and biogeographic data can deepen our understanding of species evolution and adaptive responses to environmental change (Kremer et al., 2012).

In this methodological context and because *Manilkara* studies have applied heterogeneous marker systems this review briefly clarifies the DNA-marker concepts used throughout the synthesis.

DNA markers are defined as particular genomic sequences that disclose inheritable genetic variation among individuals or populations. These markers may be located within coding or non-coding regions of the genome and are identified using molecular techniques such as PCR amplification or DNA sequencing, offering indirect insights into genetic diversity and evolutionary relationships (Nilkanta et al., 2017; Amiteye, 2021). They can also be employed to identify polymorphisms among distinct genotypes within a population by examining variations in specific DNA sequences (Amom et al., 2018; Bidyananda et al., 2024).

This review focuses on the application of molecular characterization to elucidate patterns of genetic diversity, population structure, and phylogenetic relationships among species of the genus *Manilkara*. Specifically, we: (i) identify molecular markers employed in previous studies; (ii) summarize the diversity of Manilkara species investigated and (iii) synthesize methodological gaps and future directions for ensuring the resilience of the species.

This global framework is particularly relevant, because in Peru, *Manilkara bidentata* is classified as a threatened species under Supreme Decree No. 043-2006-AG. This status requires the integration of molecular information to answer key questions about genetics, phylogeny, connectivity, and sampling representativeness. That is why has motivated us to synthesize the diversity of molecular studies conducted worldwide on species of the genus *Manilkara*. In parallel, in Peru we are developing genomic analyses and a reconstruction of the actual spatial distribution of *M. bidentata* (Contract N° PE501093163-2024-PROCIENCIA-BM). In general, our objective is to provide the global academic community dedicated to molecular studies for research on the conservation and improvement of species of the genus *Manilkara*, optimize the allocation of resources, promote the integration of research with policies, support countries in adapting their forest governance strategies, and contribute to the sustainable development of the world's forests.

This document is organized as follows: Section 2 describes materials and methods; Section 3 describes the results; Section 4 describes the discussions; Section 5 describes future directions; and Section 6 describes the conclusions.

2. Materials and Methods

2.1. Study Design

As there is still no comprehensive review addressing the molecular characterization of *Manilkara* species, this study was developed as an exploratory synthesis. It aims to gather and evaluate available scientific evidence to (i) identify the molecular markers used in prior research; (ii) analyze the diversity of *Manilkara* species examined while highlighting major taxonomic, methodological, and geographic gaps in current molecular studies and (iii) outline future methodological directions to advance molecular applications and improve the adaptive and resilience potential of these species amid global environmental challenges.

2.2. Bibliographic Search and Selection of Articles

A literature search was performed in Scopus scientific database, using Boolean combinations such as. "*Manilkara*" AND "Molecular characterization", "*Manilkara*" AND "molecular markers", "*Manilkara*" AND "genetic diversity", "*Manilkara*" AND "population structure", "*Manilkara*" AND "genetic", "*Manilkara*" AND "phylogeny" OR "phylogenetics". The selection of articles was carried out in four steps. First, elimination of duplicate studies. Second, review of titles and abstracts according to inclusion and exclusion criteria. Third, review of relevant citations in Scopus to identify additional studies. Fourth, we prioritized studies reporting extractable metrics, but we did not exclude studies solely for lacking uniform metrics; instead, such studies were synthesized narratively.

Scopus was selected as the primary source for document retrieval due to its broad coverage of peer-reviewed literature and its standardized indexing and export functionalities, which facilitate a transparent and reproducible screening workflow (Burnham, 2006). The decision to rely on a single database stems from the formatting incompatibilities that often arise when combining datasets from multiple sources (Scott et al., 2021). A comparative search conducted in both Scopus and Web of Science (WoS) the two leading academic indexing platforms (Chadegani et al., 2013) showed that Scopus contained 53% more records, with 85% of the WoS entries already included in Scopus. Therefore, Scopus was selected as the most suitable database for this study.

The literature search and data collection were carried out until December 31, 2025, the date established as the cutoff date for inclusion of studies in this review.

2.3. Eligibility Criteria

2.3.1. Inclusion Criteria

The studies were selected based on the following criteria. (i) articles that considered species of the genus *Manilkara* as the object of study; (ii) research that used molecular markers for genetic and phylogenetic characterization. (iii) Studies published in peer-reviewed scientific journals; (iii) Articles in English and (v) research published between 1999 and 2025, to cover the historical development of molecular techniques applied to this genus.

2.3.2. Exclusion Criteria

The following types of studies were excluded. (i) articles that did not use molecular markers for species of the genus *Manilkara*; (ii) research that did not focus on species of the genus *Manilkara* and (iii) non-peer-reviewed gray literature (theses, technical reports, posters or conference abstracts).

2.4. Study Selection

A structured search yielded a total of 47 articles (Figure 1). After applying manual screening according to the established inclusion and exclusion criteria, 23 studies were ultimately retained, ensuring a focused and detailed review. All data were sourced exclusively from the Scopus database.

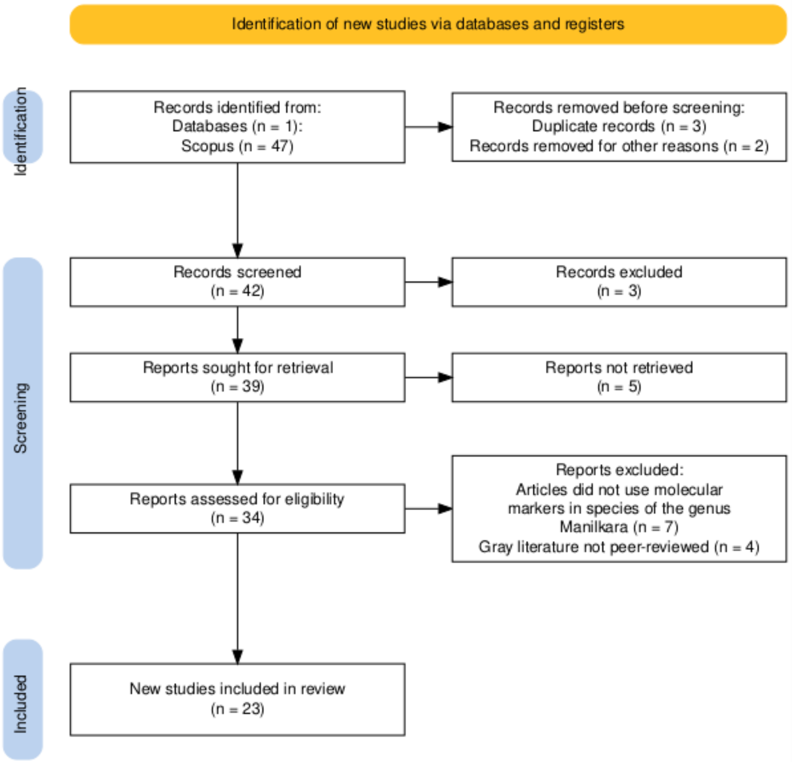

Figure 1. PRISMA-style flow diagram (reporting structure) illustrating the steps followed in the search, selection, and screening of studies included in this exploratory review. Adapted from Haddaway et al. (2022).

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2.5. Data Extraction

From studies that met the inclusion criteria, we extracted: (i) general information (authors, year, title, and journal); (ii) methodological characteristics (molecular marker type(s), sample size, and geographic scope); (iii) main outcomes, including genetic diversity metrics, population structure/differentiation, phylogenetic or evolutionary inferences (when reported), and any evidence of gene flow or introgression (when assessed) and (iv) reported methodological limitations and research gaps. Throughout this review, we use “individuals” for sampled organisms within populations, “accessions” for germplasm entries or other curated sampling units, and “populations” for geographically defined sampling groups as described in the original studies.

3. Results

3.1. Study Characteristics

Of the 23 publications selected, six publications belong to Q1 quartile journals, representing 26%. Nine publications belong to Q2 quartile journals, representing 39%. Five publications belong to Q3 quartile journals, representing 22%. Three publications belong to Q4 quartile journals, representing 13%. The main publishers identified were Wiley-Blackwell Publishing Ltd (2), Elsevier B.V. (2), Oxford University Press (2), Springer Nature (2), University of Brawijaya (2) and Cambridge University Press (2) (See Supplementary Table S1).

Also, Supplementary Figure S1 shows the evolution over time (1999-2025) of the use of different molecular markers in studies on species of the genus *Manilkara*. It can be seen that SSRs are the most widely used marker, with sustained use in almost all periods since 2005. cpDNA has been used intermittently, with applications in 2007, 2013, 2018, and 2020. RAPD appears only three times (1999, 2013, and 2017), indicating occasional use. ITS is used in specific years (2014 and 2023), without continuity. cpSSR has been used in the year 2008. SCoT has been used in the year 2020. Overall, the trend shows a growing and sustained preference for SSR as the main marker, while the others are used sporadically.

Of these, seven studies were from Brazil (Azevedo et al., 2008, 2007, 2005; Moraes et al., 2013; Sebbenn et al., 2008; Silva-Junior et al., 2016; Waqar et al., 2021a), five from the United States (Arias et al., 2020; Armstrong et al., 2014; Ganzhorn et al., 2015; Heaton et al., 1999; Thompson et al., 2015), four from India (Goraniya et al., 2013; Malik et al., 2013; Rathva et al., 2024; Sathanandam et al., 2022), two of Mexico (González-Hernández et al., 2012; Martínez-Natarén et al., 2017), two from Indonesia (Sari and Murti, 2015; Sari et al., 2018), one from Thailand (Vanijajiva, 2020), one from China (Liu et al., 2019) and one from Switzerland (Randriarisoa et al., 2023) (Figure 2).

Figure 2. Heat map showing the frequency of scientific output on molecular markers in species of the genus *Manilkara* by country.

Likewise, within the studies, Figure 3 indicates a strong dominance of SSR markers (13/23 records), used mainly in Brazil (6), followed by the USA (3), India (2), and Mexico (2). RAPD is the second most frequent marker (5/23), reported primarily in India and Indonesia (2 each) and less often in the USA (1). All other markers are rare: SCoT appears only once (Thailand), while cpDNA/cpSSR and ITS are infrequent and typically reported in combination (China, Brazil, Switzerland, and the USA).

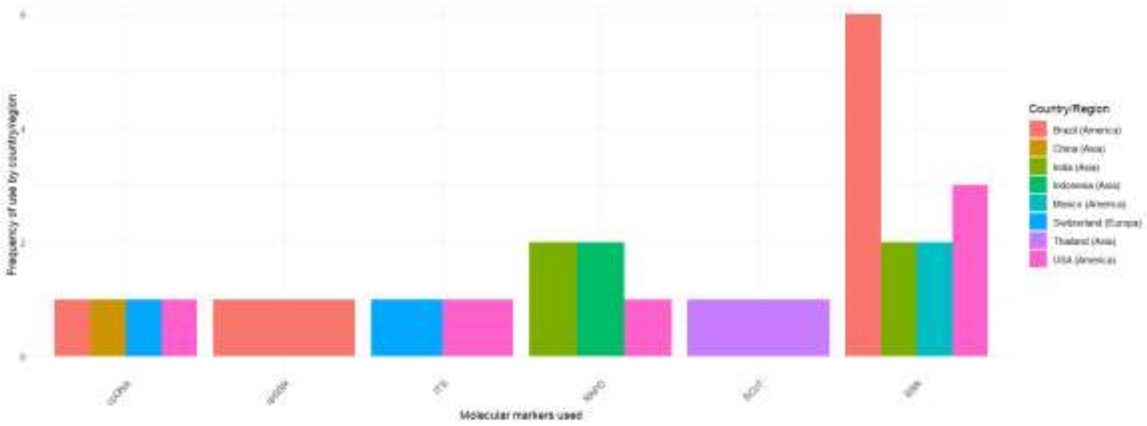

Figure 3. Frequency of molecular marker use by country/region in studies of species within the genus *Manilkara*.

3.2. Synthesis of the Results

The review of the scientific articles showed that markers can be classified into two groups, based on the type of molecular marker used: (i) RAPD, SCoT, nuSSR, and cpSSR markers, for main studies of genetic diversity and population structure (Heaton et al., 1999; Azevedo et al., 2005; 2007; 2008; Sebbenn et al., 2008; González-Hernández et al., 2012; Moraes et al., 2013; Goraniya et al., 2013; Malik et al., 2013; Thompson et al., 2015; Ganzhorn et al., 2015; Sari and Murti, 2015; Silva-Junior et al., 2016; Martínez-Natarén et al., 2017; Sari et al., 2018; Arias et al., 2020; Vanijajiva, 2020; Waqar et al., 2021; Sathanandam et al., 2022; Rathva et al., 2024) and (ii) nuclear ITS markers and cpDNA-derived markers used in phylogeny and evolutionary studies of the genus *Manilkara* (Armstrong et al., 2014; Liu et al., 2019; Randriarisoa et al., 2023). Therefore, we reviewed the studies separately according to the type of molecular approach used.

3.3. Utilization of Molecular Marker Systems

The key features of the studies included in this review are presented in Supplementary Table S2. Five studies used on RAPDs as the markers of choises (Heaton et al., 1999; Malik et al., 2013; Goraniya et al., 2013; Sari and Murti, 2015; Sari et al., 2018). One study applied SCoT markers (Vanijajiva, 2020). Thirteen studies used SSR markers (Arias et al., 2020; Azevedo et al., 2007, 2005; Ganzhorn et al., 2015; González-Hernández et al., 2012; Martínez-Natarén et al., 2017; Moraes et al., 2013; Rathva et al., 2024; Sathanandam et al., 2022; Sebbenn et al., 2008; Silva-Junior et al., 2016; Thompson et al., 2015; Waqar et al., 2021a). One study focused utilised cpSSR markers (Azevedo et al., 2008). One study focused on sequencing cpDNA (Liu et al., 2019). One study applied ITS nuclear markers and three more cpDNA-derived markers (Armstrong et al., 2014). Only one study used ITS nuclear markers (Randriarisoa et al., 2023). In Supplementary Table S2, for the purpose of updating the taxonomic nomenclature of the species under study, we have verified World Flora Online, which was updated in December 2024 (WFO, 2025), and Plants of the World Online (POWO, 2025).

The development of DNA markers may rely on coding or non-coding regions of genes, and depending on the technique used, prior sequence information may or may not be required (Bidyananda et al., 2024; Nadeem et al., 2018). Researchers continue to develop more reliable and accurate DNA markers, currently applying IRAP, REMAP, RBIP, IPBS, PARMS, CDDP, PBA, TBP, ISAP, S-SAP, ILPs, iSNAP, DALP, PAAP, TRAP, CoRAP, SCoT and DAMD, nuSSR, ISSR, cpSSR, ITS, SNP and DArT (Amiteye, 2021). In addition, recent sequencing methods such as Skim sequencing and PacBio HiFi, among others, offer higher resolution for detecting genomic variation and complement marker-based approaches (Wang et al., 2023; Wang (王博) et al., 2025). An optimal DNA marker should exhibit key attributes including high polymorphism, clear dominant or co-dominant inheritance, widespread occurrence, stability across environmental and developmental conditions, strong reproducibility, straightforward accessibility, and rapid, cost-effective analysis (Amom and Nongdam, 2017; Hasan et al., 2021).

3.3.1. Random Amplification of Polymorphic DNA (RAPD)

RAPDs represent one of the first-generation DNA markers that were the result of amplifying random DNA regions across various loci using short, arbitrary primers. This technique allowed the detection of polymorphisms in the amplified fragments which are visualized by electrophoresis (Williams et al., 1990; Bidyananda et al., 2024). This technique is straightforward, rapid, and inexpensive, and demonstrated strong efficiency in assessing genetic variation in both wild and cultivated plant species (Mendonça et al., 2014). Some constrains of RAPDs involved the dominant nature of these markers which limited the detection of genotype at individual-levels (because heterozygous and dominant homozygous cannot be differentiated) and it can increase sampling variance and bias in population-structure parameters, reducing the power to detect actual differentiation (Lynch and Milligan, 1994). Other studies reported lack of reproducibility as RAPD banding patterns may be less reliable due to sensitivity to reaction conditions (Amiteye, 2021). Subsequent individual genotyping using codominant or sequence-based markers would better resolve fine-scale structure and clarify potential genotype–phenotype associations. In addition, some studies using composite DNA samples from a given population (DNA bulking) seemed to affect individual resolution and dilute allele-frequency signals in pooled samples, constraining discrimination among germplasm sources (Reyes-Valdés et al., 2013).

Among the earliest molecular assessments in *Manilkara*, Heaton et al. (1999) used RAPD markers from bulked DNA samples to test whether the morphological differentiation observed between forest and swamp populations of *M. zapota* had a detectable genetic correlation. Across four populations, RAPD data indicated no significant genetic differentiation ($\chi^2 = 68.35$; $P > 0.1$), suggesting potential genetic homogeneity at the loci surveyed in the populations and/or a failure of the markers to tag regions associated to local differentiation. In this study, the combination of RAPD and bulking offered rapid, low-cost-effective screening to answer questions about differentiation u=in populations from different geographic origin.

Building on early findings, Malik et al. (2013) combined agro-morphological descriptors with RAPD markers to characterize diversity among 23 *M. hexandra* accessions from Gujarat and Madhya Pradesh. Fruits of this species have some importance in the region and understanding levels of diversity unlocked opportunities in breeding. The study generated 119 bands (78% polymorphic), with a pairwise similarity ranging from 0.52 to 0.82. The multivariate analysis showed and two main clusters, indicating appreciable diversity within the germplasm assessed. Given the study goal (germplasm screening), RAPD markers provided an informative overview of genetic differentiation.

Goraniya et al. (2013) used 25 RAPD primers to compare species *M. hexandra* with Averrhoa carambola, generating polymorphic profiles for both species, each one showing different marker profiles which were validated with UPGMA clustering based on a similarity matrix. The authors concluded here that RAPD markers can be more appropriate for identification/authentication and for similarity-based grouping rather than for inferring evolutionary phylogeny, because RAPD is a dominant marker system which prevent of additional inferences on species differentiation (Sun et al., 2024).

From a methodological standpoint, Sari and Murti (2015) addressed a practical bottleneck in the study of *Manilkara* genetics, the lack of DNA quality. At the time of the study, the current methods for DNA isolation could not separate contaminants in *Manilkara* zapota so that the DNA extracted showed low quality. This group worked on optimizing the CTAB-based DNA extraction from mature *M. zapota* leaves which were rich in polyphenols, polysaccharides and other secondary metabolites. Presence of those compounds are known to obstruct enzymatic events and negatively impact amplification of DNA. Their protocol named "Modification 5" (adapted from Doyle and Doyle (1990)) improved the recovery of DNA and made it suitable for downstream PCR, which is relevant for studies where tissue chemistry can compromise genotyping. Though this was a rather technical effort, the generation of a procedure that showed consistently yield DNA of good quality was important for the research groups doing DNA-based research in *Manikara*.

Later, Sari et al. (2018) assessed genetic diversity among 26 *M. zapota* accessions from five districts using RAPD markers. The goal of this project was to determine levels of spatial variation among the five districts assessed. The results showed that accessions tend to cluster by their location of origin. An attempt to test association between markers with fruit shape showed no correlation. This study reported high polymorphism (98.85% across 87 RAPD loci), with slightly more variation within populations (53%) than among populations (47%), and clusters that broadly reflected geographic origin; accessions collected in the Gunungkidul region showed comparatively higher diversity than the other locations, highlighting this as a good candidate area for conservation and selection.

In summary, studies conducted using RAPD markers in *Manilkara* provided preliminary insights on the levels of genetic variation for populations of *Manikara spp*. Most of these studies revealed patterns of genetic variation to detect some genetic differentiation and structure in the populations evaluated. Some inferences were also presented about strategies of conservation and breeding. Some limitations in the studies can be attributed to sampling size and an underestimation of diversity due to the use of dominant markers. But, in the past, RAPD markers were one of the most important genetic markers and many studies relied on their use.

3.3.2. Start Codon-Targeted Polymorphism (SCoT)

SCoT markers target short, conserved regions surrounding the ATG start codon in plant genes (Collard and Mackill, 2009). Compared to arbitrary RAPD and ISSR markers, they offer higher reproducibility and efficiency in analyzing genetic diversity and population structure across various plant species (Amom et al., 2020). Each SCoT primer consists of 18 nucleotides designed to anneal to the conserved sequence near the start codon, with detection performed through PCR amplification followed by visualization on ethidium bromide stained agarose gels (Panchariya et al., 2024). However, because SCoT profiles are typically scored as dominant (presence/absence bands), a direct estimation of allele frequencies and heterozygosity (Ho/He) is not straightforward without additional assumptions or dedicated estimators (Lynch and Milligan, 1994).

Building upon the methodological advantages, Vanijajiva (2020) assessed patterns of genetic diversity among 36 accessions in four *Manilkara* species (*Manilkara hexandra*, *Manilkara kauki*, *Manilkara littoralis*, and *Manilkara zapota*) in Thailand using 30 SCoT primers. The assay generated 176 bands (57.38% polymorphic), and multivariate analysis allowed to separate these accessions into two main clusters, which were consistent with locality and the morphological features evaluated such as fruit characteristics. This study supported the use of SCoT markers as a effective tool for cultivated-germplasm characterization unlocking possibilities of using them for conservation and breeding. In particular, the author of this study emphasized the advantages of using SCoT markers for effective conservation of germplasm by reducing size of collections (core collections).

3.3.3. Simple Sequence Repeats (SSRs)

Simple Sequence Repeats (SSRs), or microsatellites, are DNA markers composed of short tandemly repeated motifs whose repeat numbers vary among loci (Coutinho et al., 2018). These markers provide several advantages over other molecular systems. They are highly reproducible and do not require ultra-pure DNA templates, making them reliable for genetic analyses. Moreover, SSRs exhibit high polymorphism, enabling the detection of allelic differences even among closely related genotypes (Amom and Nongdam, 2017). Their co-dominant inheritance facilitates straightforward result interpretation, and their abundance and widespread distribution across eukaryotic genomes further enhance their utility (Tautz, 1989).

Simple sequence repeats (SSRs) are codominant and typically highly polymorphic markers that are well suited for estimating genetic diversity and related parameters such as gene flow, parentage, and population structure across species. In *Manilkara*, Azevedo et al. (2005) developed and characterized 12 microsatellite loci for *M. huberi* from an enriched genomic library. The loci were highly variable (4-8 alleles per locus) with high expected heterozygosity (He = 0.721-0.862), supporting applications in diversity assessment and sustainable management. The initial characterization relied on a small sample (n = 12), so broader testing across populations and ecological settings would strengthen inference (Scaketti et al., 2025). Expanding on this, Azevedo et al. (2007) applied SSRs to evaluate genetic structure, gene flow, and the mating system of *M. huberi*. They reported high genetic diversity (He > 0.82), a predominance of allogamy (t_m = 0.995), inbreeding (f = 0.175–0.240), and restricted pollen flow (47 m), and recommended collecting seed from at least 175 trees and conserving large continuous areas to maintain variability. Because the study used seven SSR loci, fine-scale estimates would benefit from additional markers and complementary field validation where feasible. From a modeling perspective, Sebbenn et al. (2008) modeled how selective logging could affect the genetic diversity and population composition of *M. huberi* and other Amazonian tree species, using genotype data at microsatellite loci for the simulations. They concluded that current practices are not sustainable for *M. huberi*, predicting drastic reductions in basal area and loss of alleles under short cycles and low minimum cutting diameters. They therefore recommended species-specific management prescriptions. As a simulation-based study, conclusions depend on assumptions about starting population conditions and disturbance history, so results are best interpreted alongside empirical monitoring.

In a related approach, González-Hernández et al. (2012) tested the transferability of *M. huberi* microsatellites to characterize genetic variation in *M. zapota*. They detected many alleles but low heterozygosity, highlighting potential genetic erosion and the need to expand sampling for conservation. Only 7 of the 12 loci were useful in *M. zapota* and null alleles may have influenced estimates; their dendrogram should therefore be viewed as similarity-based clustering rather than a phylogenetic framework.

Complementarily, Moraes et al. (2013) developed eight polymorphic microsatellites for *M. multifida*, reporting high allelic diversity and heterozygosity without evidence of null alleles, which supports conservation and genetic management applications. Sampling was restricted to two locations; therefore, broader geographic sampling is advisable before generalizing population-level patterns, as sampling design and geographic coverage strongly influence how well genetic diversity is represented in conservation-oriented inferences (Rosenberger et al., 2021).

Similarly, by using of microsatellites, Thompson et al. (2015) assessed genetic variation and structure across five *M. zapota* populations located in ancient Maya ceremonial centers, home gardens, and clonal cultivars to test whether cultivation reduced diversity. They found substantial variation and low differentiation, with no evidence of reduced diversity associated with management, consistent with strong natural gene flow in dense forests. Comparisons are limited by the absence of fully unmanaged wild populations and potential imbalance among management categories.

Following this, Ganzhorn et al. (2015) evaluated spatial genetic structure in *M. maxima* to test whether recent fragmentation reduced genetic variability. They found that fragmentation had not markedly reduced diversity and that gene flow/connectivity among populations remained high. The analysis was based on five SSR loci and low population density in fragmented sites, which can limit resolution for subtle structure. To broaden the range of available markers, Silva-Junior et al. (2016) developed 17 species-specific SSR markers for *M. maxima* and analyzed genetic diversity across three natural populations. The loci showed high variability and strong discriminatory capacity, with no indication of substantial null alleles, supporting their use in population genetics and ecological research. The survey covered three sites and did not explicitly evaluate among-population differentiation, so expanded spatial coverage would strengthen inference (Wang et al., 2024).

In a landscape-level study, Martínez-Natarén et al. (2017) used SSRs to analyze genetic diversity and population structure of *M. zapota* in 15 forest fragments and one continuous forest to evaluate long-term fragmentation. They reported high diversity and low differentiation, with little effect of fragment size or isolation. The analysis relied on three SSR loci and an unreplicated continuous-forest reference, limiting detection of fine-scale structure (Wang et al., 2021; Alves et al., 2024). From a technological standpoint, Arias et al. (2020) generated a nuclear SSR resource for *M. zapota* to support germplasm conservation and population genetics, obtaining 3,870 primer pairs and evaluating 384 loci to identify 47 high-quality polymorphic SSRs. This substantially expands tools for fingerprinting and diversity screening. Sampling per group was small (4-10 accessions) and excluded natural populations, and loci were not coupled to population-structure or trait-based analyses, so downstream validation across wild and managed materials remains important.

Subsequently, Waqar et al. (2021b) compared adults and juveniles of *M. multifida* in two protected fragments to evaluate intergenerational impacts of fragmentation. They found a marked reduction in heterozygosity and greater genetic structure in juveniles, consistent with reduced gene flow under habitat loss. The study used eight SSR loci and two sites (the Veracel Station (EVC) and the Una Biological Reserve (UBR)), and paternity-based inferences may be sensitive to incomplete sampling of reproductive adults.

More recently, Sathanandam et al. (2022) developed 30 highly polymorphic SSR markers for *M. zapota* and analyzed 53 genotypes. They detected high genetic diversity and differentiation, identifying three main groups with strong admixture, providing useful tools for conservation and genetic improvement. Interpretation is bounded by the ex-situ focus and limited phenotypic or environmental data to explain genetic patterns, and technical replication was not clearly specified.

Finally, Rathva et al. (2024) analyzed 10 genotypes of *M. achras* (currently *M. zapota* (L.) P. Royen, according to POWO (2025) using 20 SSRs. They detected 59 alleles and moderate-to-high genetic diversity, with grouping by geographical origin and alleles useful for genetic fingerprinting, supporting varietal identification and breeding. The relatively small sample size may yield imprecise or biased estimates and thus limits broader population-level inference Scaketti et al. (2025). In addition, the absence of standard diversity and structure metrics further constrains comparability across studies.

3.3.4. Chloroplast Simple Sequence Repeat (cpSSRs) Microsatellites

Microsatellites, also known as SSRs, are dispersed randomly throughout both nuclear and chloroplast genomes in plants. Compared to nuclear genomes, chloroplast genomes tend to have a lower mutation rate, which makes it more challenging to detect significant sequence variation within them (Amiteye, 2021). In contrast, cpSSR usually exhibit lower levels of polymorphism than nuSSRs due to the conserved nature of chloroplast genomes. However, they remain valuable for tracing maternal inheritance, seed dispersal, and phylogeographic patterns in plants (Moore et al., 2010; Drew et al., 2014). The non-coding regions of cpDNA - primarily introns and intergenic spacers - evolve at a slow rate and tend to be highly conserved within species. Despite this, they are particularly useful for phylogenetic and phylogeographic research, as they provide insight into maternal inheritance patterns and evolutionary connections among populations or species (Shaw et al., 2007). Interest in these regions stems from the generally slow evolutionary rate of cpDNA, which limits its variability for population and evolutionary analyses. Moreover, non-coding regions often consist of repetitive DNA sequences arranged in tandem, such as microsatellites (Dong et al., 2012). Although cpDNA exhibits low allelic diversity, it remains a valuable tool for reconstructing phylogenetic relationships among closely related taxa and exploring maternal genetic structure (Moore et al., 2010; Haasl and Payseur, 2011; Drew et al., 2014). Overall, cpDNA has proven to be an informative marker system, enabling the generation of robust data for elucidating phylogenetic relationships both within and between species (Moore et al., 2010; Drew et al., 2014).

Building on this principle, Azevedo et al. (2008) used cpSSRs together with cpDNA sequences to test for matrilineal spatial genetic structure in *M. huberi*, that is, patterns expected under limited seed-mediated dispersal. They detected significant spatial structure up to 250 m and identified 15 chloroplast haplotypes, indicating high chloroplast diversity despite modest haplotype richness. The authors interpreted these patterns as consistent with isolation by distance and argued for conserving large, contiguous tracts of primary forest to maintain locally structured maternal lineages. Because the study focused on a single population and only four of nine cpSSR loci were polymorphic, the inferred scale of structure should be viewed as population-specific. Chloroplast inheritance was treated as strictly maternal, although occasional nonmaternal (including paternal) chloroplast inheritance has been reported in some angiosperms (McCauley et al., 2007).

In a complementary approach, Liu et al. (2019) assembled and annotated the complete chloroplast genome (159,853 bp; 125 genes) of *M. zapota* to clarify its phylogenetic placement within Sapotaceae. The plastome provides a genomic resource for comparative studies and, in their cpDNA-based tree, *Manilkara* clustered with other Ericales lineages, appearing close to Ebenaceae representatives included in the analysis. However, the inference relied on a single accession and plastid-only data, which limits the ability to evaluate intraspecific variation and to detect reticulate processes; plastid and nuclear datasets may also be incongruent due to factors such as hybridization and shared ancestral polymorphism (López-Alvarado et al., 2014). In addition, the use of neighbor-joining with limited taxon sampling (13 species) constrains phylogenetic resolution; broader sampling and likelihood/Bayesian frameworks, ideally combined with nuclear markers, would strengthen inference.

3.3.5. Internal Transcribed Spacer (ITS)

When it comes to identifying species accurately, ITS markers are essential tools in DNA barcoding. These markers work by targeting the spacer DNA found within the repetitive sequences of ribosomal RNA genes (Chen et al., 2010). What makes the ITS region of nuclear ribosomal DNA particularly useful is its interesting dual nature: it shows considerable variation between different species, yet remains relatively stable within any given species. This unique characteristic is precisely why researchers favor these genetic markers when they need to distinguish species from one another (Cheng et al., 2016).

In terms of phylogeny, the genus *Manilkara* was reconstructed using nuclear and plastid sequences to infer its origin and dispersal. It was determined that, using a relaxed molecular clock calibrated with fossils, *Manilkara* evolved between 32 and 29 million years ago (Mya) in Africa. This was followed by two dispersal events, to tropical America (26-18 Mya) and tropical Asia (28-15 Mya). Higher rates of diversification were identified in the Neotropical clade, confirming that the pantropical distribution is due to long-distance dispersal events, not continental fragmentation (Armstrong et al., 2014).

The Maximum Clade Credibility (MCC) tree obtained from the BEAST analysis (Figure 7) indicates that the subtribe Manilkarinae has an average estimated age of about 32 million years, with a Highest Posterior Density (HPD) interval ranging from 36 to 29 Mya (node K). The genus *Manilkara* dates to approximately 29 million years ago (HPD 32–28 Mya; node Q), suggesting that both lineages originated during the Oligocene period. Moreover, the findings show that speciation and intercontinental dispersal events within *Manilkara* took place from the Oligocene through the Miocene, becoming particularly pronounced from the middle to late Miocene.

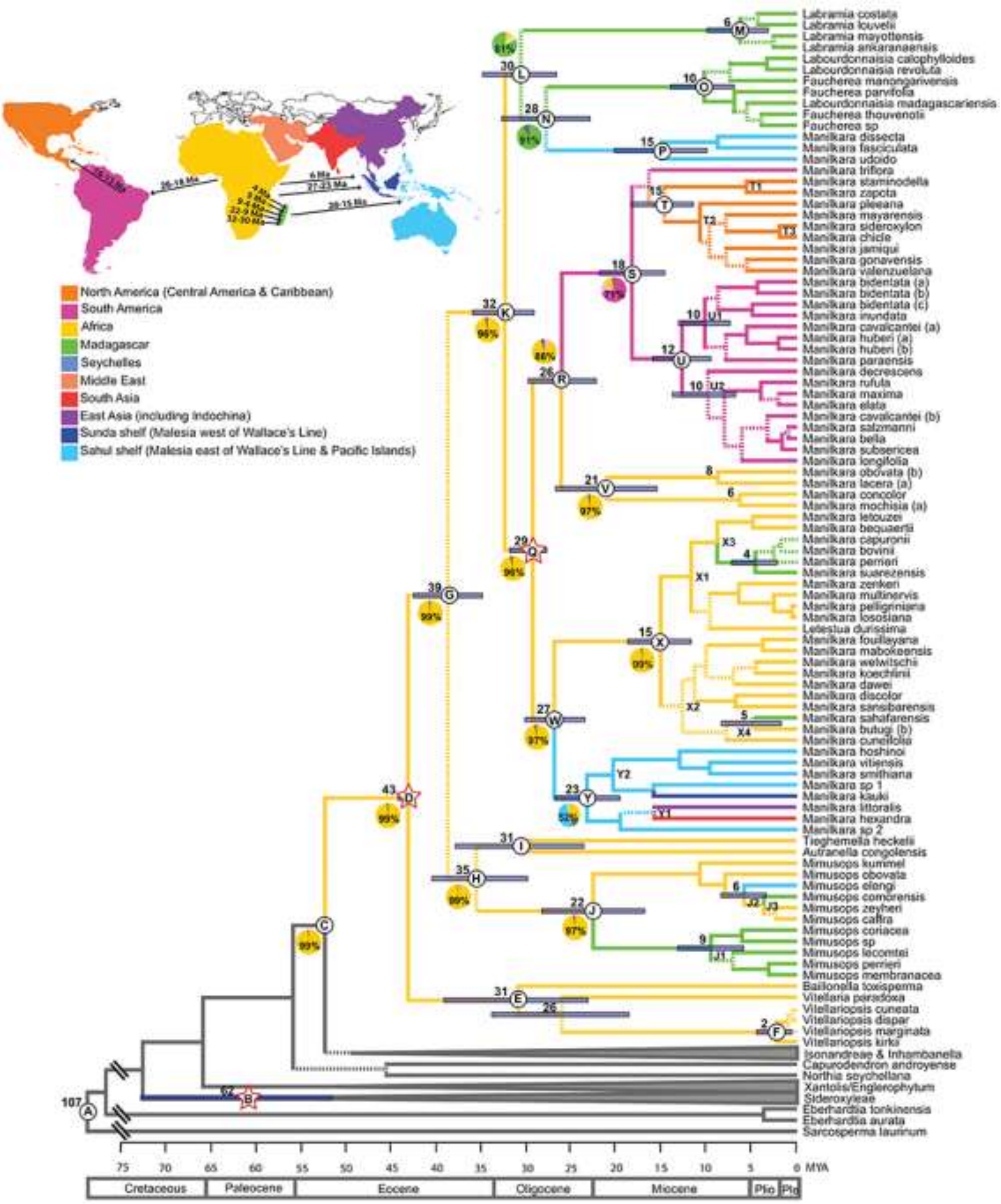

Figure 4. Time-calibrated phylogeny and biogeographic structure of *Manilkara* and related genera (ITS dataset). Figure reproduced from Armstrong et al. (2014). *Frontiers in Genetics*, 5:362. Licensed under CC BY 4.0.

The maximum clade credibility chronogram derived from the ITS dataset depicts the temporal diversification and continental distribution of *Manilkara* within the tribe Mimusopeae (Sapotaceae). The genus originated in Africa during the Oligocene (29 Mya) and subsequently dispersed to other tropical regions through long-distance colonization events.

Four principal regional lineages of *Manilkara* are resolved: (i) African clades (X, V): represent the basal and most ancestral groups, showing diversification from the Oligocene to the Miocene, including both rainforest and dry-adapted taxa. (ii) Neotropical clade (S): derived from an African ancestor between 26 and 18 Mya, and later differentiated into South American (U2) and Central American-Caribbean (T) subclades during the Miocene (16-10 Mya). (iii) Asian clade (Y): originated from a single African dispersal between 27 and 19 Mya, with limited subsequent radiation across the

Sahul and Sunda shelves. (iv) Madagascan lineages (X3-X4): represent at least two independent colonization events from Africa between 8 and 4 Mya.

Branch colors correspond to continental regions - Africa (yellow), Madagascar (green), Asia (blue), South America (pink), and Central America & Caribbean (orange). The bars at each node represent the 95% Highest Posterior Density (HPD) intervals, which show the estimated range of divergence times. Pie charts represent ancestral-area probabilities at key nodes, and the inset map summarizes the timing and direction of intercontinental dispersal inferred from the chronogram.

Expanding on these phylogenetic efforts, Randriarisoa et al. (2023) determined the genetic variability and phylogenetic structure of several Amazonian species of *Manilkara* using ITS and cpDNA sequences. It found relatively limited haplotype diversity and marked genetic differentiation between species and populations. The authors conclude that *Manilkara*, as currently defined, is not monophyletic, and they recommend a taxonomic revision. Floral evolution provides useful synapomorphies for redefining genera. The effectiveness of phylogenomic in resolving complex relationships in groups with morphological convergence is highlighted.

The phylogenetic analysis of Manilkarinae reveals a clearly defined structure composed of two principal clades (Figure 8). The first clade (PP = 0.99) groups *Labramia* and *Manilkara* s.str. as sister lineages, while the second clade (PP = 1.00) includes the three Pacific *Manilkara* species, which form a distinct lineage, as also reported by Armstrong et al. (2014), sister to the clade that encompasses all *Labourdonnaisia* and *Faucherea* species.

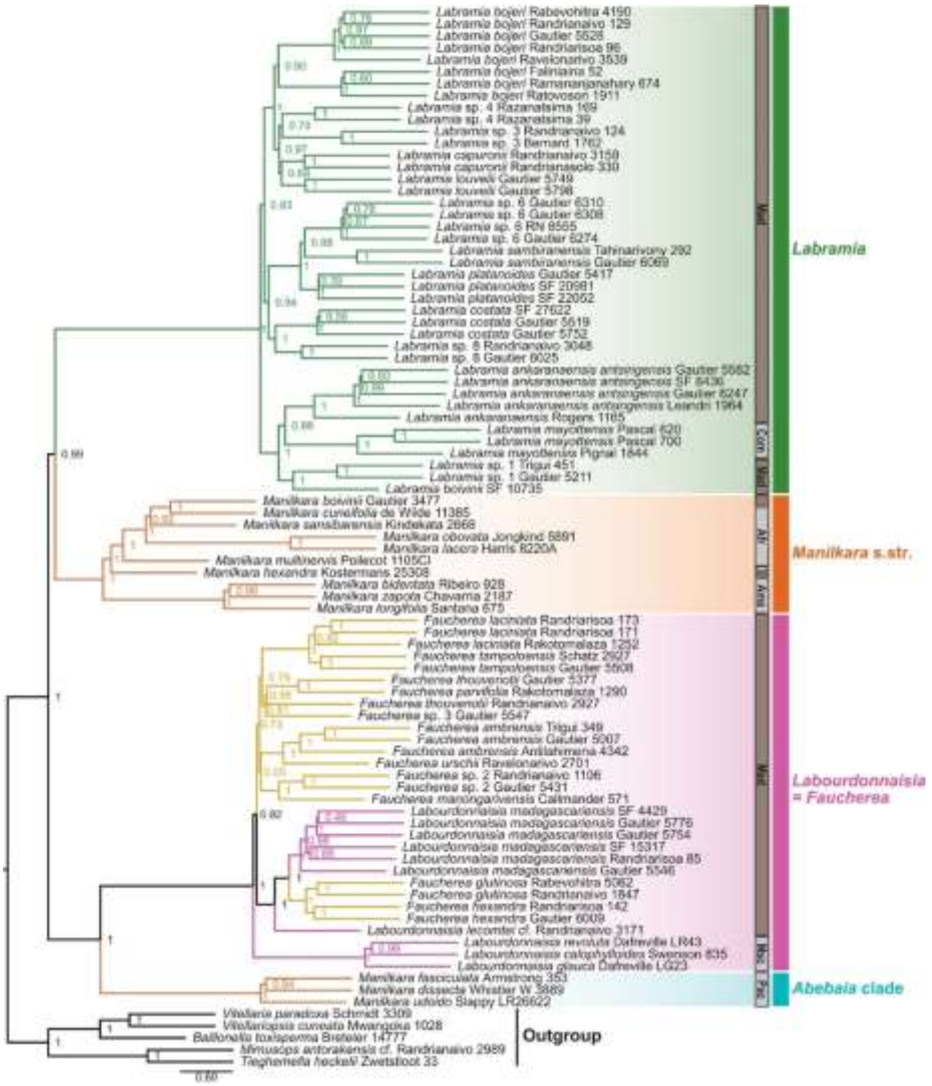

Figure 5. Species tree reconstruction of *Manilkara* and related genera inferred from ASTRAL-II. Figure reproduced from Randriarisoa et al. (2023). *Taxon*, 72(6):1404-1428. Licensed under CC BY 4.0.

The species tree generated with ASTRAL-II, using data from 89 specimens and 787 gene trees reconstructed with RAxML, elucidates the phylogenetic relationships among species of *Manilkara* and related genera within the tribe Mimusopeae (Sapotaceae). The topology supports four major, well-defined genetic clades that partly correspond to the traditional taxonomic classification.

Within *Manilkara*, the core lineage (*Manilkara* s.str.) (orange) forms a strongly supported monophyletic group closely related to the Labourdonnaisia-Faucherea clade (pink), both representing the main diversification centers of the genus across Africa, Madagascar, and the Neotropics. The *Labramia* clade (dark green) appears as a basal lineage within Mimusopeae, mostly restricted to Madagascar and the Mascarene Islands, confirming its early divergence from *Manilkara* s.str. In contrast, the *Abebaia* clade (blue) is recovered as a distinct evolutionary lineage, clearly separated from *Manilkara*, with affinities to the African - Asian complex.

ASTRAL support values are shown for internal nodes. Because ASTRAL estimates only internal branch lengths, terminal branches are represented with equal length for all taxa. Branch colors correspond to generic circumscription: *Labramia* (dark green), *Manilkara* s.str. (orange), *Labourdonnaisia* + *Faucherea* (pink), and *Abebaia* (blue). Regional abbreviations: Afr - Africa; Mad - Madagascar; Msc - Mascarenes; Com - Comoros; Ind - Indonesia; Pac - Pacific Asia; Ame - Americas; RN - Réserves Naturelles; SF - Service Forestier.

4. Discussion

Using an exploratory synthesis, we integrated genetic and phylogenetic evidence for *Manilkara* from 23 peer-reviewed studies published between 1999 and 2025. Grouping studies by marker system and geographic scope revealed substantial progress in characterizing genetic diversity, population structure, and lineage relationships, but also exposed persistent geographic, methodological, and taxonomic gaps that currently limit conservation inference. In particular, research effort is concentrated in the Americas and Asia, whereas African lineages (despite Africa being inferred as the center of origin for *Manilkara*) remain markedly underrepresented (Armstrong et al., 2014). This imbalance is consistent with broader constraints in Sapotaceae systematics, including cryptic diversity, morphological complexity, limited high-quality herbarium material, and a shortage of recent integrative taxonomic work (Christe et al., 2021).

Verification of *Manilkara* presence records in Global Biodiversity Information Facility (GBIF.org, 2026) revealed a highly uneven spatial distribution of sampling effort and data mobilization, rather than a faithful representation of the genus's true distribution. This verification produced a dataset of 39,701 records (See Supplementary Figure S2). Accordingly, GBIF occurrence records for *Manilkara* reflect a biased pattern of data availability, which increases uncertainty when assessing population status, genetic representativeness, and climate-change vulnerability in poorly documented regions. From a conservation perspective, priorities include: (i) targeted surveys and sampling in underrepresented areas where *Manilkara* is expected to occur, (ii) strengthening inventories and collections with reliable georeferencing and metadata, and (iii) designing genomic sampling campaigns that maximize spatial and environmental coverage, avoiding inference of "absences" from data gaps.

Across species, comparisons between phenotypic differentiation and genetic structure suggest that morphology does not always track neutral genetic variation. Forest versus swamp populations of *M. zapota* show pronounced phenotypic differences, yet RAPD and SSR data indicate low to moderate genetic differentiation (Heaton et al., 1999; González-Hernández et al., 2012; Martínez-Natarén et al., 2017), implying that environmentally induced plasticity may contribute strongly to observed morphological divergence. By contrast, *M. huberi* shows high heterozygosity and maintained reproductive performance under selective logging, consistent with demographic stability and sustained gene flow buffering genetic erosion (Azevedo et al., 2007; Sebbenn et al., 2008). Intermediate diversity has been reported for Atlantic Forest endemics (e.g., *M. multifida* and *M. maxima*), where restricted dispersal and fragmentation can constrain connectivity, particularly if pollen-mediated gene flow is reduced. For several Asian taxa (e.g., *M. hexandra* and *M. littoralis*), the evidence base remains comparatively weak because studies have relied largely on dominant PCR-based markers with limited allelic resolution. Collectively, these patterns motivate integrative designs that combine genome-wide data with standardized morphometrics and eco-physiological measurements to distinguish adaptive divergence from plastic responses across *Manilkara*.

Taxonomic coverage is itself a major limitation. The literature is dominated by a small subset of commercially important or accessible species (notably *M. huberi*, *M. zapota*, and *M. multifida*), whereas many wild or poorly known taxa remain genetically uncharacterized (Christe et al., 2021; Wei and Jiang, 2021; Phang et al., 2025). This restricts phylogenetic completeness and undermines the identification of evolutionarily significant units (ESUs) that could guide conservation prioritization (Hoelzel, 2023). Addressing this gap requires deliberate sampling strategies that target underrepresented regions and lineages (especially African taxa) and that link vouchers, geo-referenced metadata, and open sequence resources to reduce downstream uncertainty.

Methodological heterogeneity further limits comparability across studies. Variation in DNA extraction protocols, locus choice, marker system, diversity metrics, and sampling design impedes cross-study synthesis and can bias conclusions about diversity, structure, and connectivity. The predominance of neutral markers (e.g., SSR, RAPD) has been valuable for first-pass characterization but is limited for testing hypotheses about selection, functional variation, and climate-related adaptation (Kremer et al., 2012; Savolainen et al., 2013; Wei and Jiang, 2021; Feng et al., 2024).

A cross-cutting limitation is the weak integration of genetic evidence with ecological and landscape information, which restricts inference about how environmental gradients, fragmentation, and land-use history shape genetic variability (Dauphin et al., 2023; Aitken et al., 2024; Chambers et al., 2025). Only a small subset of studies explicitly incorporates fragmentation context, mating systems, or pollen and seed dispersal processes, despite their centrality for interpreting diversity and connectivity in tree populations. In *M. zapota*, for example, SSR-based studies illustrate how conclusions regarding fragmentation effects or potential domestication signals can shift with sampling design and marker resolution (Thompson et al., 2015; Martínez-Natarén et al., 2017), consistent with general expectations about fragmentation-driven genetic responses in plants (Aguilar et al., 2008).

In the context of accelerating climate change and biodiversity loss (Feng et al., 2024; Li et al., 2025), future research should prioritize coordinated, question-driven sampling that quantifies intraspecific diversity and phenotypic plasticity across environmental gradients, while expanding geographic and taxonomic coverage. Combining nuclear and plastid evidence (e.g., ITS, cpDNA, and additional nuclear loci) with ecological and morphological datasets should improve phylogenetic resolution and clarify evolutionary relationships. Establishing systematic collections, seed banks, and germplasm repositories spanning Neotropical, African, and Asian regions will provide the baseline required for comparative and conservation genomics and for subsequent breeding-oriented applications (Chung et al., 2023; Heuertz et al., 2023; Aitken et al., 2024; Alseekh et al., 2025).

Recent phylogenetic work underscores that taxonomic instability in *Manilkara* is closely tied to morphological homogeneity, the scarcity of diagnostic characters, and long-standing controversies in species delimitation (Randriarisoa et al., 2023). Across Manilkarinae, revisions of generic boundaries and the adoption of phylogenomic datasets have become increasingly necessary because trees inferred from few loci often lack resolution and may not capture complex evolutionary histories (Randriarisoa et al., 2023; Swenson et al., 2023; Beckers et al., 2025). Although ITS-based analyses (especially when interpreted alongside plastid and additional nuclear loci) have improved biogeographic and phylogenetic inference, restricted taxon sampling and continued reliance on single or few loci still constrain robust evolutionary interpretation, particularly when morphological evidence is not explicitly integrated (Christe et al., 2021; Randriarisoa et al., 2023). Moreover, if reticulate processes such as hybridization or introgression contribute to gene-tree discordance, low-locus approaches can be misleading, reinforcing the need for multilocus and genome-scale strategies (Hibbins and Hahn, 2022; Bjornson et al., 2024). Future work that couples phylogenomic inference with explicit tests of discordance will be essential to resolve intrageneric relationships and clarify the roles of speciation and adaptation in this tropical lineage (Bock et al., 2023; Olivares et al., 2024; Pezzini et al., 2023).

5. Future Directions

In *Manilkara*, evidence has been dominated by marker-based studies, while the adoption of genomic approaches is relatively recent. However, in trees and plants, genomic and multi-omic approaches allow genomic variation to be linked to stress response mechanisms and traits associated with biological fitness (Feng et al., 2024; Ijaz et al., 2024; Li et al., 2025). Integrative frameworks that combine genomic datasets with transcriptomic, epigenomic, or metabolomic layers can accelerate the identification of putatively functional variants and improve inference in non-model tropical tree genomes (Naidoo et al., 2019; Feng et al., 2024).

Reduced-representation and target-capture genotyping (GBS/GBTS) can support population-scale inference in non-model trees, once taxonomy, vouchered collections, and geographically balanced sampling are secured (Feng et al., 2024). Genomic selection and genomic prediction are increasingly operational in forest trees, but their gains depend on training-population design, cost–benefit planning, and multi-site validation—not “plug-and-play” deployment (Lebedev et al., 2020; Grattapaglia, 2022).

CRISPR/Cas has strong proof-of-concept in woody species, yet for largely wild *Manilkara* it is best framed short-term as functional validation, given regeneration bottlenecks and lengthy field testing (Cao et al., 2022; Cardi et al., 2023). Therefore, near-term priorities are foundational: reference resources, standardized marker panels, harmonized phenotyping, and ethical/benefit-sharing safeguards that enable reproducible, scalable datasets for downstream modeling (El-Kassaby et al., 2024). AI-assisted phenotyping can be realistic via UAV/ground imagery, but only when models are trained on quality-controlled data and externally validated across environments and seasons (Xu and Li, 2022).

Multi-omics and microbiome studies can clarify stress mechanisms, yet their immediate value depends on replication, experimental controls, and hypothesis-driven designs rather than broad exploratory profiling (Naidoo et al., 2019; Feng et al., 2024).

In light of new trends, where the deployment of genetically improved material is contemplated, decisions should be evaluated within ecological and socioeconomic frameworks, including regulation and public acceptance of genetically modified or genome-edited trees (Koch et al., 2025; Li et al., 2025; Spök et al., 2022). Risk assessments should explicitly consider gene escape and containment feasibility (Caradus, 2025), alongside context-dependent benefits for restoration, carbon sequestration, and sustainable agroforestry (Islam et al., 2024). As genotyping costs continue to fall, integrating genome-wide marker systems (e.g., GBS, skim sequencing, and long-read approaches) with predictive breeding and multi-omics will be increasingly important for next-generation forest tree improvement, but only when clear research questions, appropriate sampling designs, and reproducible data standards with open repositories are in place (Xu et al., 2022; Grattapaglia, 2022; Duarte et al., 2024; Mora-Poblete et al., 2024; Sthapit et al., 2025).

6. Conclusions

This review of 23 peer-reviewed studies (1999–2025) shows that molecular research in *Manilkara* has advanced from early PCR-based markers to microsatellites and, more recently, plastome and nuclear sequencing, but evidence is still insufficient to fully characterize genetic representativeness and evolutionary relationships across the genus.

Research remains strongly biased toward a few economically important and accessible species, leaving most *Manilkara* taxa genetically unassessed and limiting the delineation of conservation-relevant units, particularly in underrepresented African lineages.

Cross-study synthesis is constrained by methodological heterogeneity (sampling design, marker choice, and inconsistent reporting of quality control and core statistics), which reduces comparability and helps explain divergent diversity estimates for the same species.

Future work should prioritize broader, well-documented sampling (voucher specimens, coordinates, metadata), standardized reporting, and combined nuclear–plastid evidence to improve inference on lineage history, connectivity, and population structure.

Where feasible, genome-wide SNP datasets and targeted sequencing, supported by automated pipelines and machine-learning tools for quality control and genotype–environment analyses, should complement legacy markers to strengthen demographic and conservation inference.

Integrating genomics with ecological and landscape data, enhanced by remote sensing and AI-based spatial modeling, will improve the identification of conservation units and support effective restoration, long-term persistence, and sustainable use of *Manilkara*.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Author Contributions: Einstein Bravo: Conceptualization, Data curation, Investigation, Writing – original draft, Writing – review & editing. **Alfonso H. del Río**: Data curation, Investigation, Writing – review & editing. **Héctor V. Vásquez:** Validation, Resources, Visualization, Writing – review & editing. **Einstein Sánchez:** Data curation, Validation, Visualization, Writing – review & editing. **Omer Cruz:** Data curation, Formal analysis, Validation, Resources, Visualization, Writing – review & editing. **Eli Pariente:** Visualization, Writing – review & editing. **Rosalynn Rivera:** Conceptualization, Validation, Visualization, Writing – review & editing. **Carlos I. Arbizu:** Conceptualization, Visualization, Writing – review & editing.

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References

1. Aguilar, R., Quesada, M., Ashworth, L., Herrerias-Diego, Y., Lobo, J., 2008. Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. Molecular Ecology 17, 5177–5188. https://doi.org/10.1111/j.1365-294X.2008.03971.x
2. Aitken, S.N., Jordan, R., Tumas, H.R., 2024. Conserving Evolutionary Potential: Combining Landscape Genomics with Established Methods to Inform Plant Conservation. Annual Review of Plant Biology 75, 707–736. https://doi.org/10.1146/annurev-arplant-070523-044239
3. Alseekh, S., Klemmer, A., Yan, J., Guo, T., Fernie, A.R., 2025. Embracing plant plasticity or robustness as a means of ensuring food security. Nat Commun 16, 461. https://doi.org/10.1038/s41467-025-55872-4
4. Alves, S.I.A., Dantas, C.W.D., Macedo, D.B., Ramos, R.T.J., 2024. What are microsatellites and how to choose the best tool: a user-friendly review of SSR and 74 SSR mining tools. Front. Genet. 15. https://doi.org/10.3389/fgene.2024.1474611

5. Amiteye, S., 2021. Basic concepts and methodologies of DNA marker systems in plant molecular breeding. Heliyon 7. https://doi.org/10.1016/j.heliyon.2021.e08093
6. Amom, T., Nongdam, P., 2017. The use of molecular marker methods in plants: A review. Int. J. Curr. Res. Rev. 9.
7. Amom, T., Tikendra, L., Apana, N., Goutam, M., Sonia, P., Koijam, A.S., Potshangbam, A.M., Rahaman, H., Nongdam, P., 2020. Efficiency of RAPD, ISSR, iPBS, SCoT and phytochemical markers in the genetic relationship study of five native and economical important bamboos of North-East India. Phytochemistry 174, 112330. https://doi.org/10.1016/j.phytochem.2020.112330
8. Amom, T., Tikendra, L., Rahaman, H., Potshangbam, A., Nongdam, P., 2018. Evaluation of genetic relationship between 15 bamboo species of North-East India based on ISSR marker analysis. Mol Biol Res Commun 7, 7–15. https://doi.org/10.22099/mbrc.2018.28378.1303
9. Arias, R.S., Ballard, L.L., Duke, M.V., Simpson, S.A., Liu, X.F., Orner, V.A., Sobolev, V.S., Scheffler, B.E., Martinez-Castillo, J., 2020. Development of nuclear microsatellite markers to facilitate germplasm conservation and population genetics studies of five groups of tropical perennial plants with edible fruits and shoots: rambutan (Nephelium lappaceum L.), sapodilla (Manilkara zapota (L.) P. Royen), lychee (Litchi chinensis Sonn.), mangosteen (Garcinia mangostana Linn. and Garcinia cochinchinensis (Lour.) Choisy) and bamboo (Bambusa vulgaris Schrad. ex J.C. Wendl and Guadua angustifolia Kunth). Genet Resour Crop Evol 67, 1715–1731. https://doi.org/10.1007/s10722-020-00965-w
10. Armstrong, K.E., Stone, G.N., Nicholls, J.A., Valderrama, E., Anderberg, A.A., Smedmark, J., Gautier, L., Naciri, Y., Milne, R., Richardson, J.E., 2014. Patterns of diversification amongst tropical regions compared: a case study in Sapotaceae. Front. Genet. 5. https://doi.org/10.3389/fgene.2014.00362
11. Avise, J.C., 2012. Molecular Markers, Natural History and Evolution. Springer Science & Business Media.
12. Azevedo, V.C.R., Kanashiro, M., Ciampi, A.Y., Grattapaglia, D., 2007. Genetic structure and mating system of Manilkara huberi (Ducke) A. Chev., a heavily logged Amazonian timber species. Journal of Heredity 98, 646–654. https://doi.org/10.1093/jhered/esm074
13. Azevedo, V.C.R., Kanashiro, M., Grattapaglia, D., Ciampi, A.Y., 2008. CpDNA variability in Manilkara huberi, a species under sustainable management in the Brazilian Amazon. Pesquisa Agropecuaria Brasileira 43, 859–867. https://doi.org/10.1590/s0100-204x2008000700010
14. Azevedo, V.C.R., Vinson, C.C., Ciampi, A.Y., 2005. Twelve microsatellite loci in Manilkara huberi (Ducke) Standl (Sapotaceae), an Amazonian timber species. Molecular Ecology Notes 5, 13–15. https://doi.org/10.1111/j.1471-8286.2004.00815.x
15. Beckers, V., Jepma, P.J., Kokshoorn, S., Wilkie, P., Hendriks, K., Smets, E., Lens, F., 2025. Wood anatomy helps solve challenging taxonomic relationships in the tropical Sapotaceae. https://doi.org/10.1163/22941932-bja10198
16. Betts, M.G., Wolf, C., Pfeifer, M., Banks-Leite, C., Arroyo-Rodríguez, V., Ribeiro, D.B., Barlow, J., Eigenbrod, F., Faria, D., Fletcher, R.J., Hadley, A.S., Hawes, J.E., Holt, R.D., Klingbeil, B., Kormann, U., Lens, L., Levi, T., Medina-Rangel, G.F., Melles, S.L., Mezger, D., Morante-Filho, J.C., Orme, C.D.L., Peres, C.A., Phalan, B.T., Pidgeon, A., Possingham, H., Ripple, W.J., Slade, E.M., Somarriba, E., Tobias, J.A., Tylianakis, J.M., Urbina-Cardona, J.N., Valente, J.J., Watling, J.I., Wells, K., Wearn, O.R., Wood, E., Young, R., Ewers, R.M., 2019. Extinction filters mediate the global effects of habitat fragmentation on animals. Science 366, 1236–1239. https://doi.org/10.1126/science.aax9387
17. Bidyananda, N., Jamir, I., Nowakowska, K., Varte, V., Vendrame, W.A., Devi, R.S., Nongdam, P., 2024. Plant Genetic Diversity Studies: Insights from DNA Marker Analyses. International Journal of Plant Biology 15, 607–640. https://doi.org/10.3390/ijpb15030046
18. Bjornson, S., Verbruggen, H., Upham, N.S., Steenwyk, J.L., 2024. Reticulate evolution: Detection and utility in the phylogenomics era. Mol Phylogenet Evol 201, 108197. https://doi.org/10.1016/j.ympev.2024.108197
19. Bock, D.G., Cai, Z., Elphinstone, C., González-Segovia, E., Hirabayashi, K., Huang, K., Keais, G.L., Kim, A., Owens, G.L., Rieseberg, L.H., 2023. Genomics of plant speciation. Plant Comm 4. https://doi.org/10.1016/j.xplc.2023.100599
20. Burnham, J.F., 2006. Scopus database: a review. Biomedical Digital Libraries 3, 1. https://doi.org/10.1186/1742-5581-3-1

21. Cao, H.X., Vu, G.T.H., Gailing, O., 2022. From Genome Sequencing to CRISPR-Based Genome Editing for Climate-Resilient Forest Trees. International Journal of Molecular Sciences 23, 966. https://doi.org/10.3390/ijms23020966
22. Caradus, J.R., 2025. Is co-existence and/or containment of genetically modified plants possible, and is it important? New Zealand Journal of Agricultural Research 68, 1329–1380. https://doi.org/10.1080/00288233.2025.2479724
23. Cardi, T., Murovec, J., Bakhsh, A., Boniecka, J., Bruegmann, T., Bull, S.E., Eeckhaut, T., Fladung, M., Galovic, V., Linkiewicz, A., Lukan, T., Mafra, I., Michalski, K., Kavas, M., Nicolia, A., Nowakowska, J., Sági, L., Sarmiento, C., Yıldırım, K., Zlatković, M., Hensel, G., Van Laere, K., 2023. CRISPR/Cas-mediated plant genome editing: outstanding challenges a decade after implementation. Trends in Plant Science 28, 1144–1165. https://doi.org/10.1016/j.tplants.2023.05.012
24. Carvalho, C. da S., Ballesteros-Mejia, L., Ribeiro, M.C., Côrtes, M.C., Santos, A.S., Collevatti, R.G., 2017. Climatic stability and contemporary human impacts affect the genetic diversity and conservation status of a tropical palm in the Atlantic Forest of Brazil. Conserv Genet 18, 467–478. https://doi.org/10.1007/s10592-016-0921-7
25. Chadegani, A.A., Salehi, H., Yunus, M.M., Farhadi, H., Fooladi, M., Farhadi, M., Ebrahim, N.A., 2013. A Comparison between Two Main Academic Literature Collections: Web of Science and Scopus Databases. Asian Social Science 9, p18. https://doi.org/10.5539/ass.v9n5p18
26. Chambers, E.A., Bishop, A.P., Wang, I.J., 2025. Individual-based landscape genomics for conservation: An analysis pipeline. Mol Ecol Resour 25, e13884. https://doi.org/10.1111/1755-0998.13884
27. Chen, S., Yao, H., Han, J., Liu, C., Song, J., Shi, L., Zhu, Y., Ma, X., Gao, T., Pang, X., Luo, K., Li, Y., Li, X., Jia, X., Lin, Y., Leon, C., 2010. Validation of the ITS2 Region as a Novel DNA Barcode for Identifying Medicinal Plant Species. PLOS ONE 5, e8613. https://doi.org/10.1371/journal.pone.0008613
28. Cheng, T., Xu, C., Lei, L., Li, C., Zhang, Y., Zhou, S., 2016. Barcoding the kingdom Plantae: new PCR primers for ITS regions of plants with improved universality and specificity. Molecular Ecology Resources 16, 138–149. https://doi.org/10.1111/1755-0998.12438
29. Choi, Y., Ha, Y.-H., Choi, J., 2024. Genetic diversity assessment of a plant for forest restoration on the Korean Peninsula: A case study of *Lespedeza cuneata* G. Don (Fabaceae). Journal of Asia-Pacific Biodiversity 17, 35–42. https://doi.org/10.1016/j.japb.2023.10.004
30. Christe, C., Boluda, C.G., Koubínová, D., Gautier, L., Naciri, Y., 2021. New genetic markers for Sapotaceae phylogenomics: More than 600 nuclear genes applicable from family to population levels. Molecular Phylogenetics and Evolution 160, 107123. https://doi.org/10.1016/j.ympev.2021.107123
31. Chung, M.Y., Merilä, J., Li, J., Mao, K., López-Pujol, J., Tsumura, Y., Chung, M.G., 2023. Neutral and adaptive genetic diversity in plants: An overview. Front. Ecol. Evol. 11. https://doi.org/10.3389/fevo.2023.1116814
32. Collard, B.C.Y., Mackill, D.J., 2009. Start Codon Targeted (SCoT) Polymorphism: A Simple, Novel DNA Marker Technique for Generating Gene-Targeted Markers in Plants. Plant Mol Biol Rep 27, 86–93. https://doi.org/10.1007/s11105-008-0060-5
33. Coutinho, J.P., Carvalho, A., Martín, A., Lima-Brito, J., 2018. Molecular characterization of Fagaceae species using inter-primer binding site (iPBS) markers. Mol Biol Rep 45, 133–142. https://doi.org/10.1007/s11033-018-4146-3
34. Dauphin, B., Rellstab, C., Wüest, R.O., Karger, D.N., Holderegger, R., Gugerli, F., Manel, S., 2023. Re-thinking the environment in landscape genomics. Trends in Ecology & Evolution 38, 261–274. https://doi.org/10.1016/j.tree.2022.10.010
35. De Faria, A.D., Pirani, J.R., Ribeiro, J.E.L.D.S., Nylinder, S., Terra-Araujo, M.H., Vieira, P.P., Swenson, U., 2017. Towards a natural classification of Sapotaceae subfamily Chrysophylloideae in the Neotropics. Botanical Journal of the Linnean Society 185, 27–55. https://doi.org/10.1093/botlinnean/box042
36. Dirzo, R., Raven, P.H., 2003. Global State of Biodiversity and Loss. Annual Review of Environment and Resources 28, 137–167. https://doi.org/10.1146/annurev.energy.28.050302.105532

37. Dong, Y., Yao, H., Lin, Z., Zhang, L., Bao, Z., 2012. Characterization of 62 polymorphic EST-SSR markers in the blood clam (Tegillarca granosa) and their cross-amplification in Scapharca subcrenata. Conservation Genet Resour 4, 991–997. https://doi.org/10.1007/s12686-012-9691-9
38. Doyle, J.J., Doyle, J.L., 1990. Isolation of plant DNA from fresh tissue, Focus. ed.
39. Drew, B.T., Ruhfel, B.R., Smith, S.A., Moore, M.J., Briggs, B.G., Gitzendanner, M.A., Soltis, P.S., Soltis, D.E., 2014. Another Look at the Root of the Angiosperms Reveals a Familiar Tale. Syst Biol 63, 368–382. https://doi.org/10.1093/sysbio/syt108
40. Duarte, D., Jurcic, E.J., Dutour, J., Villalba, P.V., Centurión, C., Grattapaglia, D., Cappa, E.P., 2024. Genomic selection in forest trees comes to life: unraveling its potential in an advanced four-generation Eucalyptus grandis population. Front. Plant Sci. 15. https://doi.org/10.3389/fpls.2024.1462285
41. El-Kassaby, Y.A., Cappa, E.P., Chen, C., Ratcliffe, B., Porth, I.M., 2024. Efficient genomics-based ‘end-to-end’ selective tree breeding framework. Heredity 132, 98–105. https://doi.org/10.1038/s41437-023-00667-w
42. Feng, J., Dan, X., Cui, Y., Gong, Y., Peng, M., Sang, Y., Ingvarsson, P.K., Wang, J., 2024. Integrating evolutionary genomics of forest trees to inform future tree breeding amid rapid climate change. Plant Communications 5, 101044. https://doi.org/10.1016/j.xplc.2024.101044
43. Ganzhorn, S.M., Thomas, W.W., Gaiotto, F.A., Lewis, J.D., 2015. Spatial genetic structure of Manilkara maxima (Sapotaceae), a tree species from the Brazilian Atlantic forest. Journal of Tropical Ecology 31, 437–447. https://doi.org/10.1017/S0266467415000292
44. Gautier, L., 2003. Sapotáceas. En: Goodman, JP, Bensted, SM (Ed.). La historia natural de Madagascar., The University of Chicago Press, Chicago. ed.
45. Gautier, L., Lachenaud, O., van der Burgt, X., Kenfack, D., 2016. Five new species of Englerophytum K. Krause (Sapotaceae) from central Africa, Candollea. ed.
46. GBIF.org, 2026. Global Biodiversity Information Facility [WWW Document]. URL https://doi.org/10.15468/dl.4dcg3e (accessed 2.24.26).
47. Gentry, A.H., 1993. A field guide to the families and genera of woody plants of northeast South America, Conservation International. ed. Washington, D.C.
48. González-Hernández, D., García-Pérez, E., Guntin-Marey, P., 2012. Genetic characterization of Manilkara zapota from veracruz, Mexico, with SSR markers. Agrociencia 46, 663–675.
49. Goraniya, S., Tusamda, N., Shirolkar, A.R., Rao, G., Murthy, S.N., Pawar, S.D., 2013. Molecular analysis of Manilkara hexandra Roxb. and Averrhoa carambola using RAPD markers helps to understand genetic variations. International Journal of Pharmacy and Pharmaceutical Sciences 5, 626–628.
50. Govaerts, R., Frodin, D.G., Pennington, T.D., 2001. World Checklist and Bibliography of Sapotaceae. Royal Botanic Gardens.
51. Grattapaglia, D., 2022. Twelve Years into Genomic Selection in Forest Trees: Climbing the Slope of Enlightenment of Marker Assisted Tree Breeding. Forests 13, 1554. https://doi.org/10.3390/f13101554
52. Haasl, R.J., Payseur, B.A., 2011. Multi-locus inference of population structure: a comparison between single nucleotide polymorphisms and microsatellites. Heredity 106, 158–171. https://doi.org/10.1038/hdy.2010.21
53. Haddaway, N.R., Page, M.J., Pritchard, C.C., McGuinness, L.A., 2022. PRISMA2020: An R package and Shiny app for producing PRISMA 2020-compliant flow diagrams, with interactivity for optimised digital transparency and Open Synthesis. Campbell Systematic Reviews 18, e1230. https://doi.org/10.1002/cl2.1230
54. Hasan, N., Choudhary, S., Naaz, N., Sharma, N., Laskar, R.A., 2021. Recent advancements in molecular marker-assisted selection and applications in plant breeding programmes. Journal of Genetic Engineering and Biotechnology 19, 128. https://doi.org/10.1186/s43141-021-00231-1
55. Heaton, H.J., Whitkus, R., Gómez-Pompa, A., 1999. Extreme ecological and phenotypic differences in the tropical tree chicozapote (Manilkara zapota (L.) P. Royen) are not matched by genetic divergence: a random amplified polymorphic DNA (RAPD) analysis. Molecular Ecology 8, 627–632. https://doi.org/10.1046/j.1365-294x.1999.00616.x
56. Heuertz, M., Carvalho, S.B., Galindo, J., Rinkevich, B., Robakowski, P., Aavik, T., Altinok, I., Barth, J.M.I., Cotrim, H., Goessen, R., González-Martínez, S.C., Grebenc, T., Hoban, S., Kopatz, A., McMahon, B.J., Porth, I., Raeymaekers, J.A.M., Träger, S., Valdecantos, A., Vella, A., Vernesi, C., Garnier-Géré, P., 2023. The

application gap: Genomics for biodiversity and ecosystem service management. Biological Conservation 278, 109883. https://doi.org/10.1016/j.biocon.2022.109883

57. Hibbins, M.S., Hahn, M.W., 2022. Phylogenomic approaches to detecting and characterizing introgression. Genetics 220, iyab173. https://doi.org/10.1093/genetics/iyab173
58. Hoelzel, A.R., 2023. Where to now with the evolutionarily significant unit? Trends in Ecology & Evolution 38, 1134–1142. https://doi.org/10.1016/j.tree.2023.07.005
59. Hollingsworth, P.M., Graham, S.W., Little, D.P., 2011. Choosing and Using a Plant DNA Barcode. PLOS ONE 6, e19254. https://doi.org/10.1371/journal.pone.0019254
60. Ijaz, S., Iqbal, J., Abbasi, B.A., Yaseen, T., Rehman, S., Kazi, M., Ansari, L., Mahmood, T., 2024. Role of OMICS-Based Technologies in Plant Sciences, in: OMICs-Based Techniques for Global Food Security. pp. 45–66. https://doi.org/10.1002/9781394209156.ch3
61. Islam, K.K., Saifullah, M., Mahboob, M.G., Jewel, K.N.-E.-A., Ashraf, S.M.K., Hyakumura, K., 2024. Restoring Soil Fertility, Productivity and Biodiversity through Participatory Agroforestry: Evidence from Madhupur Sal Forest, Bangladesh. Land 13, 326. https://doi.org/10.3390/land13030326
62. Kassaye, M., Emiru, E., Derebe, Y., Tsega, A., 2025. Carbon stock dynamics in Ethiopian forests; a systematic review for sustainable forest management towards climate change mitigation. Trees, Forests and People 20, 100841. https://doi.org/10.1016/j.tfp.2025.100841
63. Koch, M., DeMond, J., Pence, M.G., Schaefer, E.A., Rudgers, G., 2025. One risk assessment for genetically modified plants. Front. Bioeng. Biotechnol. 13. https://doi.org/10.3389/fbioe.2025.1619857
64. Kremer, A., Ronce, O., Robledo-Arnuncio, J.J., Guillaume, F., Bohrer, G., Nathan, R., Bridle, J.R., Gomulkiewicz, R., Klein, E.K., Ritland, K., Kuparinen, A., Gerber, S., Schueler, S., 2012. Long-distance gene flow and adaptation of forest trees to rapid climate change. Ecology Letters 15, 378–392. https://doi.org/10.1111/j.1461-0248.2012.01746.x
65. Kümpers, B.M.C., Richardson, J.E., Anderberg, A.A., Wilkie, P., Ronse De Craene, L.P., 2016. The significance of meristic changes in the flowers of Sapotaceae. Botanical Journal of the Linnean Society 180, 161–192. https://doi.org/10.1111/boj.12363
66. Laurance, W.F., Sayer, J., Cassman, K.G., 2014. Agricultural expansion and its impacts on tropical nature. Trends in Ecology & Evolution 29, 107–116. https://doi.org/10.1016/j.tree.2013.12.001
67. Lebedev, V.G., Lebedeva, T.N., Chernodubov, A.I., Shestibratov, K.A., 2020. Genomic Selection for Forest Tree Improvement: Methods, Achievements and Perspectives. Forests 11, 1190. https://doi.org/10.3390/f11111190
68. Li, C., Li, B., Zhao, W., Jiang, J., Tang, J., 2025. Forest tree breeding under the global environmental change: Challenges and opportunities. Trees, Forests and People 20, 100867. https://doi.org/10.1016/j.tfp.2025.100867
69. Liu, J., Ren, S.-N., Li, K.-X., Xu, T., Niu, Y.-F., Shi, C., 2019. The complete chloroplast genome sequence of Manilkara zapota (Linn.) van Royen. Mitochondrial DNA Part B 4, 2127–2128. https://doi.org/10.1080/23802359.2019.1623122
70. López-Alvarado, J., Sáez, L., Filigheddu, R., Garcia-Jacas, N., Susanna, A., 2014. The limitations of molecular markers in phylogenetic reconstruction: The case of Centaurea sect. Phrygia (Compositae). TAXON 63, 1079–1091. https://doi.org/10.12705/635.6
71. Lynch, M., Milligan, B.G., 1994. Analysis of population genetic structure with RAPD markers. Molecular Ecology 3, 91–99. https://doi.org/10.1111/j.1365-294X.1994.tb00109.x
72. Malik, S.K., Kumar, S., Choudhary, R., Kole, P.R., Chaudhury, R., Bhat, K.V., 2013. Assessment of genetic diversity in khirni [Manilkara hexandra (Roxb.) Dubard]: An important underutilized fruit species of India using random amplified polymorphic DNA markers. Indian Journal of Horticulture 70, 18–25.
73. Martínez-Natarén, D.A., Parra-Tabla, V., Munguía-Rosas, M.A., 2017. Genetic diversity and structure of the tree Manilkara zapota in a naturally fragmented tropical forest. Journal of Tropical Ecology 33, 285–294. https://doi.org/10.1017/S0266467417000268
74. McCauley, D.E., Sundby, A.K., Bailey, M.F., Welch, M.E., 2007. Inheritance of chloroplast DNA is not strictly maternal in Silene vulgaris (Caryophyllaceae): evidence from experimental crosses and natural populations. American Journal of Botany 94, 1333–1337. https://doi.org/10.3732/ajb.94.8.1333

75. Mendonça, E.G., de Souza, A.M., Vieira, F. de A., Estopa, R.A., Reis, C.A.F., Carvalho, D. de, 2014. Using Random Amplified Polymorphic DNA to Assess Genetic Diversity and Structure of Natural Calophyllum brasiliense (Clusiaceae) Populations in Riparian Forests. International Journal of Forestry Research 2014, 305286. https://doi.org/10.1155/2014/305286
76. Moore, M.J., Soltis, P.S., Bell, C.D., Burleigh, J.G., Soltis, D.E., 2010. Phylogenetic analysis of 83 plastid genes further resolves the early diversification of eudicots. Proceedings of the National Academy of Sciences 107, 4623–4628. https://doi.org/10.1073/pnas.0907801107
77. Moraes, R.C.S., Vivas, C.V., Oliveira, F.A., Menezes, I.P.P., van den Berg, C., Gaiotto, F.A., 2013. Microsatellite markers for an endemic Atlantic Forest tree, Manilkara multifida (Sapotaceae). AoB PLANTS 5, plt006. https://doi.org/10.1093/aobpla/plt006
78. Mora-Poblete, F., Mieres-Castro, D., Amaral Júnior, A.T. do, Balach, M., Maldonado, C., 2024. Integrating deep learning for phenomic and genomic predictive modeling of *Eucalyptus* trees. Industrial Crops and Products 220, 119151. https://doi.org/10.1016/j.indcrop.2024.119151
79. Munzinger, J., Swenson, U., 2009. Three new species of Planchonella Pierre (Sapotaceae) with a dichotomous and online key to the genus in New Caledonia, Adansonia, séries 3. ed.
80. Nadeem, M.A., Nawaz, M.A., Shahid, M.Q., Doğan, Y., Comertpay, G., Yıldız, M., Hatipoğlu, R., Ahmad, F., Alsaleh, A., Labhane, N., Özkan, H., Chung, G., Baloch, F.S., 2018. DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. Biotechnology & Biotechnological Equipment 32, 261–285. https://doi.org/10.1080/13102818.2017.1400401
81. Naidoo, S., Slippers, B., Plett, J.M., Coles, D., Oates, C.N., 2019. The Road to Resistance in Forest Trees. Front. Plant Sci. 10. https://doi.org/10.3389/fpls.2019.00273
82. Nilkanta, H., Amom, T., Tikendra, L., Rahaman, H., Nongdam, P., 2017. ISSR Marker Based Population Genetic Study of Melocanna baccifera (Roxb.) Kurz: A Commercially Important Bamboo of Manipur, North-East India. Scientifica 2017, 3757238. https://doi.org/10.1155/2017/3757238
83. Olivares, I., Tusso, S., José Sanín, M., de La Harpe, M., Loiseau, O., Rolland, J., Salamin, N., Kessler, M., Shimizu, K.K., Paris, M., 2024. Hyper-Cryptic radiation of a tropical montane plant lineage. Molecular Phylogenetics and Evolution 190, 107954. https://doi.org/10.1016/j.ympev.2023.107954
84. Panchariya, D.C., Dutta, P., Ananya, Mishra, A., Chawade, A., Nayee, N., Azam, S., Gandham, R.K., Majumdar, S., Kushwaha, S.K., 2024. Genetic marker: a genome mapping tool to decode genetic diversity of livestock animals. Front Genet 15, 1463474. https://doi.org/10.3389/fgene.2024.1463474
85. Pennington, T., 2007. Flora of Ecuador 80. 152. Sapotaceae, Gothenburg: Department of Plant and Environmental Sciences, Göteborg University. ed.
86. Pennington, T., 2006. Flora da Reserva Ducke, Amazonas, Brasil: Sapotaceae, Rodriguésia. ed.
87. Pennington, T.D., 2008. Sapotaceae. In: Nuevo catálogo de la flora vascular de venezuela (Hokche, 0., P. Berry & O. Huber, eds.), Dr. Tobías Lasser. ed. Fundación Instituto Botánico de Venezuela. Caracas.
88. Pennington, T.D., 1991. The genera of Sapotaceae. New York Botanical Garden e Kew., United Kingdom.
89. Pennington, T.D., 1990. Flora Neotropica Monograph 52 Sapotaceae. New York Botanical Garden for the Organization for Flora Neotropica, New York, USA.
90. Pezzini, F.F., Ferrari, G., Forrest, L.L., Hart, M.L., Nishii, K., Kidner, C.A., 2023. Target capture and genome skimming for plant diversity studies. Appl Plant Sci 11, e11537. https://doi.org/10.1002/aps3.11537
91. Phang, A., Niissalo, M.A., Herrera-Alsina, L., Pezzini, F.F., Hollands, R., Burslem, D.F.R.P., Ruhsam, M., Middleton, D.J., Wilkie, P., Khew, G.S., 2025. Novel phylogenomic and biogeographic insights into *Palaquium* and tribe Isonandreae (Sapotaceae). Molecular Phylogenetics and Evolution 212, 108403. https://doi.org/10.1016/j.ympev.2025.108403
92. Phillips, H.R.P., Newbold, T., Purvis, A., 2017. Land-use effects on local biodiversity in tropical forests vary between continents. Biodivers Conserv 26, 2251–2270. https://doi.org/10.1007/s10531-017-1356-2
93. Pither, R., Shore, J.S., Kellman, M., 2003. Genetic diversity of the tropical tree Terminalia amazonia (Combretaceae) in naturally fragmented populations. Heredity 91, 307–313. https://doi.org/10.1038/sj.hdy.6800299
94. POWO, 2025. Plants of the World Online [WWW Document]. Plants of the World Online. URL https://powo.science.kew.org/ (accessed 7.9.25).

95. Randriarisoa, A., Naciri, Y., Armstrong, K., Boluda, C.G., Dafreville, S., Pouchon, C., Gautier, L., 2023. One in, one out: Generic circumscription within subtribe Manilkarinae (Sapotaceae). TAXON 72, 98–125. https://doi.org/10.1002/tax.12863
96. Rathva, H., Pandey, A.K., Suthar, K., Suthar, H., Chakote, A., Singh, Diwakar, Ahlawat, T., Parmar, V., Dhiman, V.K., Pandey, H., Singh, Devendra, 2024. Genetic relatedness analysis in sapota using SSR markers. Ecological Genetics and Genomics 31, 100234. https://doi.org/10.1016/j.egg.2024.100234
97. Reyes-Valdés, M.H., Santacruz-Varela, A., Martínez, O., Simpson, J., Hayano-Kanashiro, C., Cortés-Romero, C., 2013. Analysis and Optimization of Bulk DNA Sampling with Binary Scoring for Germplasm Characterization. PLOS ONE 8, e79936. https://doi.org/10.1371/journal.pone.0079936
98. Rosenberger, K., Schumacher, E., Brown, A., Hoban, S., 2021. Proportional sampling strategy often captures more genetic diversity when population sizes vary. Biological Conservation 261, 109261. https://doi.org/10.1016/j.biocon.2021.109261
99. Sari, V.K., Murti, R.H., 2015. AN EFFECTIVE METHOD FOR DNA EXTRACTION OF MATURE LEAF OF SAPODILLA (Manilkara zapota (L.) van Royen). AGRIVITA Journal of Agricultural Science 37, 18–23. https://doi.org/10.17503/agrivita.v37i1.451
100. Sari, V.K., Wulandari, R.A., Murti, R.H., 2018. Study on Diversity of Sapodilla (Manilkara zapota) by Molecular Marker in the Special Region of Yogyakarta. AGRIVITA Journal of Agricultural Science 40, 295–303. https://doi.org/10.17503/agrivita.v40i2.925
101. Sathanandam, P.N., Patil, P., Rekha, A., Muthuvel, I., Patel, A.R., Boggala, R.B., Shirol, A.M., Ravishankar, K.V., 2022. Development and characterization of microsatellite markers, genetic diversity and population structure analysis in Sapota (Manilkara zapota (L.) P. Royen). Genet Resour Crop Evol 69, 2787–2801. https://doi.org/10.1007/s10722-022-01399-2
102. Savolainen, O., Lascoux, M., Merilä, J., 2013. Ecological genomics of local adaptation. Nat Rev Genet 14, 807–820. https://doi.org/10.1038/nrg3522
103. Scaketti, M., Sujii, P.S., Alves-Pereira, A., Schwarcz, K.D., Francisconi, A.F., Moro, M.S., Martins, K.K.M., Jesus, T.A. de, Souza, G.B.F. de, Zucchi, M.I., 2025. Sample Size Impact (SaSii): An R script for estimating optimal sample sizes in population genetics and population genomics studies. PLOS ONE 20, e0316634. https://doi.org/10.1371/journal.pone.0316634
104. Scott, D.J., Broyd, T., Ma, L., 2021. Exploratory literature review of blockchain in the construction industry. Automation in Construction 132, 103914. https://doi.org/10.1016/j.autcon.2021.103914
105. Sebbenn, A.M., Degen, B., Azevedo, V.C.R., Silva, M.B., de Lacerda, A.E.B., Ciampi, A.Y., Kanashiro, M., Carneiro, F. da S., Thompson, I., Loveless, M.D., 2008. Modelling the long-term impacts of selective logging on genetic diversity and demographic structure of four tropical tree species in the Amazon forest. Forest Ecology and Management 254, 335–349. https://doi.org/10.1016/j.foreco.2007.08.009
106. Sembada, A.A., Faizal, A., Sulistyawati, E., 2024. Photosynthesis efficiency as key factor in decision-making for forest design and redesign: A systematic literature review. Ecological Frontiers 44, 1128–1139. https://doi.org/10.1016/j.ecofro.2024.07.008
107. Shaw, J., Lickey, E.B., Schilling, E.E., Small, R.L., 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. American Journal of Botany 94, 275–288. https://doi.org/10.3732/ajb.94.3.275
108. Silva-Junior, J.A., de Souza França, D., Moraes, R.C.S., Gaiotto, F.A., 2016. Development of microsatellite markers for Manilkara maxima T.D. Penn. (Sapotaceae) and their use in conservation genetics. Mol Biol Rep 43, 451–455. https://doi.org/10.1007/s11033-016-3981-3
109. Sosa, C.C., Arenas, C., García-Merchán, V.H., 2023. Human Population Density Influences Genetic Diversity of Two Rattus Species Worldwide: A Macrogenetic Approach. Genes 14, 1442. https://doi.org/10.3390/genes14071442
110. Spök, A., Sprink, T., Allan, A.C., Yamaguchi, T., Dayé, C., 2022. Towards social acceptability of genome-edited plants in industrialised countries? Emerging evidence from Europe, United States, Canada, Australia, New Zealand, and Japan. Front Genome Ed 4, 899331. https://doi.org/10.3389/fgeed.2022.899331

111. Sthapit, S.R., Crain, J., Larson, S., Anderson, J.A., Bajgain, P., DeHaan, L.R., Poland, J., 2025. A low-coverage skim-sequencing and imputation pipeline for genomic selection. Plant Genome 18, e70139. https://doi.org/10.1002/tpg2.70139
112. Swenson, U., Anderberg, A.A., 2005. Phylogeny, character evolution, and classification of Sapotaceae (Ericales). Cladistics 21, 101–130. https://doi.org/10.1111/j.1096-0031.2005.00056.x
113. Swenson, U., Lepschi, B., Lowry II, P.P., Terra-Araujo, M.H., Santos, K., Nylinder, S., Alves-Araújo, A., 2023. Reassessment of generic boundaries in Neotropical Chrysophylloideae (Sapotaceae): Eleven reinstated genera and narrowed circumscriptions of Chrysophyllum and Pouteria. TAXON 72, 307–359. https://doi.org/10.1002/tax.12894
114. Swenson, U., Lowry II, P.P., Cronholm, B., Nylinder, S., 2020. Resolving the relationships of the enigmatic Sapotaceae genera Beauvisagea and Boerlagella, and the position of Planchonella suboppositifolia. TAXON 69, 998–1015. https://doi.org/10.1002/tax.12313
115. Swenson, U., Munzinger, J., 2016. Five new species and a systematic synopsis of Pycnandra (Sapotaceae), the largest endemic genus in New Caledonia, Australian Systematic Botany. ed.
116. Swenson, U., Munzinger, J., 2012. Revision of Pichonia (Sapotaceae) in New Caledonia. Australian Systematic Botany.
117. Swenson, U., Munzinger, J., Bartish, I., 2007. Molecular phylogeny of Planchonella (Sapotaceae) and eight new species from New Caledonia, Taxon. ed.
118. Tautz, D., 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Research 17, 6463–6471. https://doi.org/10.1093/nar/17.16.6463
119. Taylor, P.D., Fahrig, L., Henein, K., Merriam, G., 1993. Connectivity Is a Vital Element of Landscape Structure. Oikos 68, 571–573. https://doi.org/10.2307/3544927
120. Thompson, K.M., Culley, T.M., Zumberger, A.M., Lentz, D.L., 2015. Genetic variation and structure in the neotropical tree, Manilkara zapota (L) P. Royen (Sapotaceae) used by the ancient Maya. Tree Genetics & Genomes 11, 40. https://doi.org/10.1007/s11295-015-0867-9
121. Vanijajiva, O., 2020. Start codon targeted (SCoT) polymorphism reveals genetic diversity of Manilkara in Thailand. Biodiversitas Journal of Biological Diversity 21. https://doi.org/10.13057/biodiv/d210232
122. Wang, H., Bernardo, A., St. Amand, P., Bai, G., Bowden, R.L., Guttieri, M.J., Jordan, K.W., 2023. Skim exome capture genotyping in wheat. The Plant Genome 16, e20381. https://doi.org/10.1002/tpg2.20381
123. Wang, Huaying, Yang, B., Wang, Huan, Xiao, H., 2021. Impact of different numbers of microsatellite markers on population genetic results using SLAF-seq data for Rhododendron species. Sci Rep 11, 8597. https://doi.org/10.1038/s41598-021-87945-x
124. Wang, Y., Ding, C., Hermoso, V., Ji, S., Du, T., Chen, J., Ding, L., Tao, J., 2024. Using Marxan to optimize the geographical and environmental representativeness of biodiversity sampling sites. Biological Conservation 294, 110591. https://doi.org/10.1016/j.biocon.2024.110591
125. Wang (王博), B., Jia (贾鹏), P., Gao (高胜寒), S., Zhao (赵焕焕), H., Zheng (郑高洋), G., Xu (许林峰), L., Ye (叶凯), K., 2025. Long and Accurate: How HiFi Sequencing is Transforming Genomics. genom. proteom. bioinform. 23, qzaf003. https://doi.org/10.1093/gpbjnl/qzaf003
126. Waqar, Z., Moraes, R.C.S., Benchimol, M., Morante-Filho, J.C., Mariano-Neto, E., Gaiotto, F.A., 2021a. Gene Flow and Genetic Structure Reveal Reduced Diversity between Generations of a Tropical Tree, Manilkara multifida Penn., in Atlantic Forest Fragments. Genes 12, 2025. https://doi.org/10.3390/genes12122025
127. Waqar, Z., Moraes, R.C.S., Benchimol, M., Morante-Filho, J.C., Mariano-Neto, E., Gaiotto, F.A., 2021b. Gene Flow and Genetic Structure Reveal Reduced Diversity between Generations of a Tropical Tree, Manilkara multifida Penn., in Atlantic Forest Fragments. Genes 12, 2025. https://doi.org/10.3390/genes12122025
128. Wei, X., Jiang, M., 2021. Meta-analysis of genetic representativeness of plant populations under ex situ conservation in contrast to wild source populations. Conservation Biology 35, 12–23. https://doi.org/10.1111/cobi.13617
129. WFO, 2025. World Flora Online [WWW Document]. URL https://www.worldfloraonline.org/ (accessed 8.12.25).

130. White, G.M., Boshier, D.H., Powell, W., 2002. Increased pollen flow counteracts fragmentation in a tropical dry forest: An example from Swietenia humilis Zuccarini. Proceedings of the National Academy of Sciences 99, 2038–2042. https://doi.org/10.1073/pnas.042649999
131. Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., Tingey, S.V., 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18, 6531–6535. https://doi.org/10.1093/nar/18.22.6531
132. Xu, R., Li, C., 2022. A Review of High-Throughput Field Phenotyping Systems: Focusing on Ground Robots. Plant Phenomics 2022. https://doi.org/10.34133/2022/9760269
133. Xu, Y., Zhang, X., Li, H., Zheng, H., Zhang, J., Olsen, M.S., Varshney, R.K., Prasanna, B.M., Qian, Q., 2022. Smart breeding driven by big data, artificial intelligence, and integrated genomic-enviromic prediction. Mol. Plant 15, 1664–1695. https://doi.org/10.1016/j.molp.2022.09.001
134. Young, A., Boyle, T., Brown, T., 1996. The population genetic consequences of habitat fragmentation for plants. Trends in Ecology & Evolution 11, 413–418. https://doi.org/10.1016/0169-5347(96)10045-8