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Advancements in Orchidaceae Species Identification: A Comprehensive Review of Traditional and Molecular Methods

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Abstract: The Orchidaceae family represents the largest and most diverse group of flowering plants or angiosperms. This family has garnered significant attention due to its aesthetic appeal, as well as its economic and ecological importance. Globally, the Orchidaceae family encompasses approximately 600-800 genera and 25,000-35,000 species. In India, the family includes 158 genera and 1,331 species. The allure and exotic beauty of orchids, combined with their high productivity, extended shelf life, optimal blooming seasons, ease of packaging and transportation, and substantial international market value, have led to frequent smuggling and illegal trade, both offline and online. Effective and accurate identification of smuggled orchid species is crucial for combating this illegal trade. The review highlights both traditional taxonomical approaches, which rely on morphological traits like floral structures, leaf morphology, and root characteristics and advanced molecular methods such as DNA barcoding, ISSR, RAPD, and SCAR markers. DNA barcoding, which employs specific DNA sequences (e.g., ITS, rbcL, and matK), enhances the accuracy of identification, particularly for species that are illegally trafficked at juvenile or sterile stages. The review also addresses the importance of precise species identification in conservation and law enforcement, which is essential for preventing illicit trade and observing international regulations such as CITES. Technical barriers in molecular methods, voids in genetic databases, and ethical concerns regarding plant conservation are examined. This review discusses the possibility of incorporating machine learning and deep learning approaches as well as the use of eDNA(Environmental DNA) for orchid identification purposes. The manuscript concludes by suggesting that additional research be conducted on portable identification technologies, AI integration, and multi-locus barcodes in order to enhance the identification of species and conservation activities, to promote sustainable conservation and prevent illegal trade. Additionally, the article explores future perspectives on the application of emerging identification techniques in this field.

Introduction

The Orchidaceae family represents the largest and most diverse group of flowering plants or angiosperms. This family has garnered significant attention due to its aesthetic appeal, as well as its economic and ecological importance. Globally, the Orchidaceae family encompasses approximately 600-800 genera and 25,000-35,000 species. In India, the family includes 158 genera

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and 1,331 species. The allure and exotic beauty of orchids, combined with their high productivity, extended shelf life, optimal blooming seasons, ease of packaging and transportation, and substantial international market value, have led to frequent smuggling and illegal trade, both offline and online. They are epiphytes and are currently experiencing high rates of extinction as a result of a variety of anthropogenic and climatic factors (Tiruwa et al., 2024). According to the State of the World's Plants and Fungi (SOTWPF), 2023 orchids are in state of peril and are threatened with extinction due to the population shrinkage in Australia (CSIRO, 2023). As orchids exhibit medicinal and pharmaceutical properties, there are numerous reports that suggest the indigenous people have extensively utilized numerous significant medicinal plant species and orchids are no exception. Additionally, pharmaceutical companies have harvested these plants for commercial purposes, posing a significant threat to their survival (Darro and Khan, 2023). Effective and accurate identification of smuggled orchid species is crucial for combating this illegal trade. Conservation and environmental management often rely on government statistics; however, there are frequent concerns regarding their accuracy and reliability, particularly when addressing sensitive issues such as unlawful harvesting and trading. Increasing evidence highlights the unrecorded illegal commercial trade of wild plants and animals (Phelps and Webb, 2015). Research and policy on illegal wildlife trade (IWT) have significantly overlooked plants. Despite numerous plant species being at risk due to illicit exploitation or illegal wildlife trade, the focus and funding dedicated to understanding, preventing, and addressing this issue predominantly neglect flora. This phenomenon, referred to as "Plant Blindness," is defined as the "misguided anthropocentric ranking of plants as inferior to animals" (Chase et al., 2015).

The botanical trade is expanding rapidly, alongside the trade in species listed under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and the International Union for Conservation of Nature (IUCN). This trend is a significant concern, as it poses a substantial threat to biodiversity and ecological balance. CITES oversees international trade in numerous species, yet trade occurring within domestic borders often escapes detection. Information on such domestic trade is typically derived from enforcement seizures or media reports, which are sporadic and fragmented (Pistoni and Toledo, 2010). Consequently, unregulated and undocumented domestic markets can directly contribute to species decline or facilitate the introduction of wildlife products into larger international trade networks.

Orchids, despite being extensively traded for purposes such as medicine, food, and horticulture, remain insufficiently studied (Hinsley and Roberts, 2017). The Orchidaceae family, comprising over 750 genera and approximately 28,000 species, is one of the most diverse families of flowering plants, with a global distribution excluding Antarctica (Chase et al., 2015). The International Union for Conservation of Nature (IUCN) Red List has assessed a limited number of orchid species, revealing concerning results: 84 out of 85 tropical Asian slipper orchids (Paphiopedilum) face imminent extinction, primarily due to commercial exploitation (IUCN, 2020). Accorcing to IUCN's global Red List assessment report in 2023, 43 new orchid species were assessed and included in the list (IUCN Orchid Specialist Group, 2023). Despite this, there is a paucity of knowledge regarding trade networks, the sustainability of harvesting practices, and the drivers of trade, which is critical given that many orchid species possess small populations, restricted habitats and are inherently vulnerable to environmental and climatic changes, making them particularly susceptible to overharvesting pressures (Koopowitz, 2001).

Orchids are prominently listed among endangered plant species, with a majority included in Appendix II of the Convention on International Trade in Endangered Species (CITES), alongside 1,601 species on the IUCN Global Red List (Ning et al., 2010; Singh et al., 2010). Although legal trade plays a vital role in supporting livelihoods in low-income countries, its sustainability is jeopardized by the ongoing illicit trade in wild-protected orchid species and their vulnerable hybrids (Hinsley and Roberts, 2017). India, home to 1,256 orchid species, of which 307 are endemic, protects only 11 species under the Wildlife (Protection) Act of 1972, highlighting the significant issue of overexploitation and illegal trafficking (Bhaduri, 2022).

Effective regulation and mitigation of this complex illicit trade network necessitate accurate identification of orchid species. It is imperative that experts and law enforcement personnel are well-equipped and skilled in various species identification techniques. A range of methodologies, from traditional taxonomical approaches to advanced molecular techniques, have been developed and continue to evolve for the precise identification of orchid species. This paper reviews existing taxonomic identification techniques, with a particular focus on DNA barcoding, which employs a single DNA segment to provide discriminatory information among living taxa.

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Materials and Methods

We used the PunMed Advanced Search Builder and Web of Science to systematically look for papers whose titles contained the words 'Orchids', 'Species identification' 'Traditional methods', 'Molecular Approach', 'Endangered and Rare orchid species', 'Environmental DNA', 'DNA Barcoding', 'Deep lerning', 'Conservation strategies'. We did not consider papers that discuss animal and human nuclear DNA. We did not limit our research by authors or author affiliations, journal or journal impact factor and by publication dates.

Taxonomical identification techniques:

Taxonomy encompasses the scientific methodology of characterizing, identifying, classifying, and naming living organisms (plants and animals) from diverse geographical regions. This discipline leverages morphological features, genetic data, and phylogenetic relationships for accurate identification.

Orchidaceae represents one of the largest families of flowering plants (Chase et al., 2015). Orchid identification and classification primarily rely on floral morphology, which also provides genetic links between species. The pedicel, located at the base of the orchid flower, serves as structural support. Sepals, leaf-like structures, are symmetrically arranged above and below the flower, while a second layer of colorful petals is positioned both above and between the sepals. Together, the sepals and petals form the perianth, which attracts pollinators despite being non-reproductive. The reproductive parts include the stamen (male organ) and the pistil (female organ). The lip or labellum, a single petal modified to serve as a landing platform for pollinators, varies in shape and color among orchid species (Dodson, 2024). Besides floral traits, leaf and stem structures also exhibit species-specific variations crucial for identification.

A study by Indraloka et al. (2019) focused on identifying Indonesian native orchid species of the Dendrobium genus based on morphological characteristics to support protection and sustainable germplasm conservation. Morphological characterization, following UPOV guidelines, was employed to gather phenotypic data. Plant morphology aids in variety identification, genetic diversity assessment, and accession differentiation (Gouda, 2020). Parameters in this study included flower, leaf, root, fruit, and labellum characteristics. Flower characterization revealed diversity in flowering positions, with variations in petal structures and racemose flower types. Leaf morphology showed diversity in shape, surface texture, tip shape, and arrangement (Pillon and Chase, 2006). The labellum, a distinctive feature of Orchidaceae, displayed significant morphological diversity.

For instance, the labellum of *Paphiopedilum rungsuriyanum* is V-shaped and brownish, while Cypripedium reginae has a white, funnel-like labellum with magenta lines (McGough et al., 2004). This characteristic is essential for orchid identification. Indraloka et al. (2019) confirmed that orchid species exhibit substantial morphological diversity in roots, leaves, and flowers, particularly the labellum.

In a study by Miswarti et al. (2021) entitled "Morphological Characteristics of Orchid Species in Bukit Barisan, Bengkulu Province," the researchers aimed to identify and characterize the orchid diversity in Bengkulu Province based on morphological characteristics. The study identified 34 orchid species from 15 genera. Observations focused on the morphological traits of leaves, flower stems, pseudobulbs, and fruit, adhering to orchid identification guidelines. Indonesia's rich biodiversity, including its vast tropical rainforests and extensive genetic resources, encompasses a wide variety of orchids. Orchids are prevalent as both epiphytes and terrestrial plants (Zhang et al., 2018).

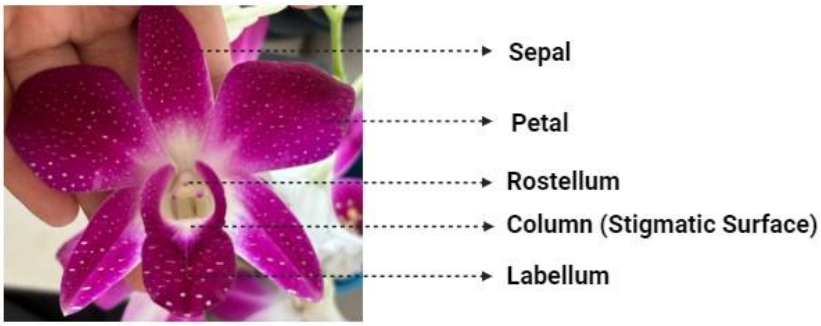

Figure 1. Typical morphological features of an orchid flower.

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The findings revealed a significant diversity among the orchids, with 34 identified species. Of these, 91.17% were epiphytic, and 8.82% were terrestrial, typically growing attached to dead or living tree branches. Additionally, the orchids exhibited two distinct growth patterns: sympodial (94.12%) and monopodial (5.8%). According to Rosanti et al. (2018), monopodial orchids are characterized by a continuously growing main stem, generally lacking pseudobulbs and rhizomes. In contrast, sympodial orchids possess a segmented main stem and pseudobulbs. Consequently, the study concluded that the identified orchid varieties have unique characteristics, such as variations in color, shape, and form of the sepals, petals, and labellum.

While morphological markers are straightforward and do not require specialized equipment, they have limitations, including their finite number, variability due to plant growth stages, and environmental influences (Eagles et al., 2001). Therefore, biochemical and molecular approaches are necessary to complement morphological findings.

Protected orchid varieties are often traded in their juvenile or sterile stages to avoid detection by enforcement officials. The market offers a range of intricate orchid varieties, which are difficult to distinguish before bloom development. Consequently, accurately identifying orchid species based on morphological traits is challenging, especially with sterile plant material. The Orchidaceae family includes many "look-alike" species, complicating differentiation without expertise. Furthermore, distinguishing whether a species falls under CITES Appendix II or Appendix I, which closely resembles protected species, adds to the difficulty (CITES, 2002; Phelps and Webb, 2015).

Biochemical markers, such as alloenzymes and isoenzymes, were initially used for genetic characterization before the advent of DNA technology (Smith, 1986; Macháčková, 1994). Phytochemical

Table 1. Different molecular loci in plants with their advantages and disadvantages.

MOLECULAR LOCI	ADVANTAGES	DISADVANTAGES
Chloroplast Genes	➢ The chloroplast genome contains genes characterized by high variability, making them valuable for genetic identification. ➢ The genome typically exhibits a high copy number within the cell, enhancing the reliability of genetic analyses. ➢ Regions of high variability within the chloroplast genome are used for fine-scale identification, such as distinguishing species and subspecies. ➢ Regions with lower variability are suitable for higher-level taxonomic identification, including the classification of genera, families, and tribes. ➢ This differential utility of genomic regions enables comprehensive and nuanced phylogenetic studies, supporting both broad and precise taxonomic resolutions. ➢ The stratified approach to genetic analysis improves the accuracy of species identification and enhances understanding of evolutionary relationships within and across various taxonomic levels. ➢ The high copy number and relative conservation of the chloroplast genome facilitate the development of robust molecular markers. ➢ These molecular markers are essential for biodiversity studies, conservation efforts, and monitoring species in ecological and commercial contexts. ➢ The use of chloroplast DNA in molecular systematics represents a critical tool in modern botanical research.	➢ Chloroplast genes typically exhibit low levels of genetic variation. ➢ This may limit their effectiveness in distinguishing closely related species or populations. ➢ Chloroplast genes are usually inherited maternally in most plants. ➢ This restricts their use in studies requiring biparental inheritance patterns. ➢ Chloroplast genes have slower mutation rates. ➢ They may not provide sufficient resolution for detecting recent evolutionary events or fine-scale population differentiation. ➢ Chloroplast genomes are small and encode a limited number of genes.

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Nuclear Genes	➢ The chloroplast genome is characterized by a high copy number per cell, enhancing the reliability and robustness of genetic analyses. ➢ It exhibits significant variability in its genes, making it highly suitable for detailed genetic identification and phylogenetic studies. ➢ The chloroplast genome demonstrates biparental inheritance, providing a comprehensive understanding of genetic contributions from both parental lineages. ➢ The high copy number within individual cells facilitates the detection and analysis of genetic material, improving the accuracy of molecular markers used in research. ➢ Substantial genetic variability allows for precise differentiation at both the species and subspecies levels, invaluable for taxonomic classification and evolutionary studies. ➢ Biparental inheritance enhances genetic diversity and provides a detailed picture of genetic transmission and variability. ➢ This inheritance pattern is particularly advantageous in studies of hybridization and gene flow, contributing to a deeper understanding of plant genetics and evolution. ➢ The attributes of the chloroplast genome make it an essential tool in modern botanical and genetic research. ➢ It aids in the conservation of biodiversity, the study of evolutionary relationships, and the advancement of molecular systematics.	➢ Sequence information from chloroplast genomes can sometimes be of poor quality, posing challenges for accurate genetic analysis and interpretation. ➢ The universal presence of chloroplast genomes across different groups of organisms increases the risk of unexpected contamination, complicating species differentiation and identification. ➢ Poor quality sequence data can hinder the resolution of phylogenetic relationships, reducing the reliability of evolutionary studies. ➢ Implementing stringent quality control measures and utilizing advanced sequencing technologies are essential to enhance data accuracy. ➢ The universal nature of chloroplast genomes necessitates meticulous experimental design and contamination control to ensure data integrity. ➢ Researchers must employ rigorous protocols to distinguish target sequences from potential contaminants, preserving the validity of findings. ➢ Addressing the challenges of poor quality sequence information and contamination risk is crucial for the effective use of chloroplast genomes in genetic and evolutionary research. ➢ Enhancing sequence quality and implementing robust contamination controls will improve the accuracy and reliability of molecular analyses. ➢ Improved practices will facilitate more precise and meaningful scientific insights in botanical and genetic research.

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Mitochondrial Genes	➢ The mitochondrial genome structure in plants, despite its rapid changes, provides unique insights into plant evolution and genetic diversity through its structural variations. ➢ The low rate of sequence change in the mitochondrial genome offers stability in genetic markers, making it useful for long-term evolutionary studies. ➢ Structural variations in the mitochondrial genome can be used to identify specific lineages and evolutionary events, offering a detailed perspective on genetic divergence. ➢ The conserved sequences within the mitochondrial genome can serve as reliable reference points for comparative genomic studies across diverse plant species. ➢ Low sequence variability ensures that any observed genetic differences are likely to be evolutionarily significant, aiding in the identification of key genetic events. ➢ The mitochondrial genome can be used to study cytoplasmic inheritance patterns, providing insights into maternal lineage and evolutionary history. ➢ Structural changes in the mitochondrial genome can act as markers for certain plant traits, contributing to the understanding of plant physiology and adaptation. ➢ The stability of certain mitochondrial genes makes them valuable for reconstructing phylogenetic relationships among plant species over long evolutionary timescales. ➢ The mitochondrial genome's unique features complement nuclear and chloroplast genomic data, offering a more comprehensive view of plant genetics and evolution when integrated.	➢ The mitochondrial genome structure in plants undergoes rapid changes, exhibiting frequent rearrangements and structural variations. ➢ Despite this structural dynamism, the mitochondrial genome exhibits a low rate of sequence change, resulting in low genetic variability. ➢ Rapid structural changes complicate the analysis of phylogenetic relationships, as rearrangements can obscure evolutionary signals. ➢ Structural instability necessitates the use of complementary genomic data to accurately interpret phylogenetic and evolutionary patterns. ➢ Low sequence variability limits the utility of the mitochondrial genome for fine-scale genetic differentiation and species identification. ➢ Researchers often rely on nuclear or chloroplast genomes for higher variability and resolution in genetic studies. ➢ Addressing the challenges posed by the plant mitochondrial genome involves integrating multiple genomic datasets. ➢ Employing advanced analytical methods is essential for a comprehensive understanding of plant genetics and evolution. ➢ These approaches help mitigate the limitations of low variability and structural instability in the mitochondrial genome. ➢

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Intergenic Spacer Regions	➢ Non-coding regions of the genome have high sequence variability, useful for genetic differentiation and evolutionary studies. ➢ These regions evolve faster than coding regions, offering a dynamic record of genetic changes over shorter evolutionary timescales. ➢ High variability aids in distinguishing closely related species and populations, enhancing phylogenetic and population genetic analyses. ➢ Rapid evolution in non-coding regions helps detect recent evolutionary events and adaptations, providing insights into species' responses to environmental changes and selective pressures. ➢ Non-coding regions serve as indicators of genetic drift, migration, and microevolutionary processes. ➢ Variability in non-coding sequences helps identify regulatory elements and non-coding RNAs, which are crucial for gene expression and regulation. ➢ Understanding these elements reveals mechanisms of gene regulation and the evolution of genetic regulatory networks. ➢ Overall, the high sequence variability and rapid evolution of non-coding regions are essential for modern genetic and evolutionary research, offering detailed insights into genetic diversity and evolutionary dynamics.	• **Intra-Species Variability:** ➢ Non-coding regions exhibit significant variability within species. ➢ This variability enhances the ability to differentiate genetic differences and explore evolutionary processes. ➢ It aids in the study of population structure, evolutionary dynamics, and adaptation mechanisms. • **Challenges in Amplification, Sequencing, and Alignment:** ➢ Non-coding regions are often difficult to amplify, sequence, and align due to high variability and repetitive elements. ➢ This complexity can lead to incomplete or biased data. ➢ Advanced methodologies and refined analytical approaches are required to address these challenges and ensure accurate genomic analyses.

analysis of orchids also aids in identification, with techniques like GC-MS, HPLC, and FTIR generating chemical profiles unique to each orchid species. However, biochemical markers may represent only a small portion of the genome and generally show low polymorphism. Molecular approaches provide extensive information, facilitating accurate species identification and characterization.

Molecular markers offer a precise approximation of genetic diversity and species identification due to their resistance to environmental factors affecting phenotype. Molecular genetic technologies have proven effective in wildlife trade monitoring for species of conservation concern (Wilson et al., 2016). Recent advancements in molecular techniques have significantly expanded our understanding of orchid genetic diversity.

DNA barcoding has emerged as a streamlined and effective method for identifying plant varieties. This technique involves analyzing specific DNA regions to distinguish species. Selecting a barcode locus requires balancing sequence substitution saturation with the need for universal applicability across taxa (Kress et al., 2005). The mitochondrial CO1 region has been effective for animal identification (Hebert et al., 2003; Shneyer, 2009). However, DNA barcoding is more complex in plants due to mitochondrial genome rearrangements, low substitution rates, and sequence imports from the nucleus and chloroplast (Wolfe et al. 1987; Drouin et al., 2008; Cho et al., 1998). Therefore, plant DNA barcoding focuses on nuclear and chloroplast genomes.

Most plant barcoding studies utilize plastid regions such as the non-coding spacer trnH-psbA, as well as core barcodes like matK, rbcL and the nuclear ribosomal DNA ITS or ITS2 regions (Kress et al., 2007; Hollingsworth et al., 2009, 2011; Li et al., 2011). While no single sequence is capable of identifying all plant species with complete accuracy, multi-locus barcoding approaches generally provide varying levels of discrimination among plant groups.

To enhance identification capabilities, additional molecular marker techniques are employed. These include Amplified Fragment Length Polymorphism

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(AFLP), Random Amplified Polymorphic DNA (RAPD), Inter-Simple Sequence Repeat (ISSR), Single Nucleotide Polymorphism (SNP), Insertion/Deletion (InDel), and Microsatellite Simple Tandem Repeat (STR) marker analyses. Each of these techniques contributes to the robustness of plant identification efforts.

Table 1 presents a comprehensive overview of the advantages and limitations associated with various molecular loci utilized in plant barcoding, highlighting their respective efficacy and application contexts.

In the study conducted by Pongsrila et al. (2017) titled "DNA Fingerprinting Analysis of Fourteen Orchid Species Using the ISSR Technique," the genetic diversity among fourteen orchid species—comprising one Ascocentrum, one Rhynchostylis, and twelve Dendrobium cultivars—was investigated using Inter-Simple Sequence Repeat (ISSR) markers. DNA extracted from these species was amplified with ten ISSR primers to evaluate genetic variation.

ISSR markers are utilized in polymerase chain reaction (PCR) amplification with primers that feature a single Simple Sequence Repeat (SSR) motif at either the 3' or 5' end, separated by a few nucleotides, with the SSRs oriented in opposing directions (Zietkiewicz et al., 1994). These markers exhibit high levels of polymorphism and are cost-effective compared to other genetic analysis methods, making them valuable for studies of genetic diversity and genome mapping (Gemmill, 2021). ISSRs are present in both organellar and nuclear genomes.

The study yielded a total of 460 ISSR fragments from the ten selected primers, including both monomorphic and polymorphic bands. Of the ten primers, eight were successful in amplifying sequences across all orchid species examined. The findings indicate that ISSRs are highly polymorphic and effective for orchid species identification, highlighting their utility in genetic and taxonomic studies.

In a study conducted by Feng et al. (2015) on the identification of Dendrobium species using the ITS2 DNA barcode region, both species identification and phylogenetic analysis were successfully achieved. The researchers utilized the ITS2 regions from 43 Dendrobium samples, which were amplified through polymerase chain reaction (PCR). The resulting sequences were aligned using Clustal W in conjunction with sequences from GenBank, and genetic distances were computed using MEGA v5.1.

The study highlights the critical conservation issue facing Dendrobium populations due to habitat destruction, overexploitation for their commercial and medicinal value, and illegal trading, which threaten their survival in natural habitats. Accurate species identification is therefore essential for effective conservation and protection efforts.

The PCR amplification and sequencing achieved a 100% success rate. The identification results, obtained through BLAST1, TaxonGAP and nearest distance methodologies, demonstrated that the ITS2 regions were effective in identifying the majority of Dendrobium species examined. Additionally, the ITS2 region proved to be a robust marker for assessing phylogenetic relationships within the genus Dendrobium. Phylogenetic analysis, conducted using the neighbor-joining (NJ) method in MEGA 5.1, provided valuable insights into the evolutionary relationships among the Dendrobium species.

DNA barcoding has been effectively employed in the species-level identification of Vanda tricolor by Su'udi et al. (2022), utilizing three primer sets: rbcL, ITS2, and matK. Following DNA isolation, the researchers performed PCR amplification and electrophoretic separation of the DNA fragments, which were subsequently sequenced. The sequence lengths obtained were 317 bp for rbcL, 461 bp for ITS2, and 408 bp for matK.

Given the critical role of floral organs in morphological identification, DNA barcoding provides a robust alternative for rapid and precise species identification, even in the absence of floral material. The validity of the selected markers was assessed using BLAST software from the NCBI database, which confirmed the effectiveness of these primers in identifying Vanda orchids. Notably, while all three primer sets were successful in identifying the species, ITS2 demonstrated superior specificity and was capable of identifying Vanda tricolor at the species level. This highlights ITS2 as the most effective marker among the ones tested for precise taxonomic classification.

In a study by Raskoti et al. (2021), DNA barcoding was applied to identify species of medicinal orchids to address the intricate network of illegal trade involving various plant parts, such as stems, pseudobulbs and tubers, used for medicinal purposes. The researchers utilized five single barcodes (ITS, ITS2, rbcL, matK and trnH-psbA) and their seven combinations, amplifying and analyzing nearly 7,000 sequences from genomic and plastid DNA.

Among the single-locus barcodes, the ITS region exhibited superior performance and the highest efficacy for identifying medicinal orchids, outperforming all other barcodes. The ITS region, a rapidly evolving nuclear

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gene with high polymorphism, significantly aids in species discrimination (Kress et al. 2005; Sass et al. 2007). In contrast, plastid barcodes such as rbcL, matK, and trnH-psbA demonstrated lower resolution compared to the ITS region.

The study also evaluated various combinations of two and three markers from ITS, ITS2, rbcL, and matK. The combination of ITS and matK (ITS + matK) provided the highest capacity for species discrimination among all combinations tested. Incorporating three-locus candidates did not significantly enhance resolution rates compared to the two-locus combinations, with their resolution remaining comparatively lower (Xiang et al. 2011; Xu et al. 2015).

In their study, Cheng et al. (2020) employed ribosomal DNA (rDNA) sequences, specifically targeting the ITS 1, 5.8S, and ITS 2 regions, to isolate and differentiate three species of Dendrobium: *Dendrobium tosaense*, *Dendrobium moniliforme*, and *Dendrobium officinale*. Following in vitro amplification of these barcode regions, the researchers observed that the genetic similarity within the rDNA regions among species pairs ranged from 91% to 95%. This study demonstrated that this rDNA-based approach is highly effective for discerning genetic variation among different Dendrobium species, providing a valuable tool for species identification and differentiation in complex taxonomic groups.

Ding et al. (2005) utilized Random Amplified Polymorphic DNA (RAPD) markers to identify eight distinct wild populations of Dendrobium officinale, focusing on molecular authentication and genetic diversity. The study employed ten decamer primers for the RAPD analysis. A DNA molecular dendrogram was constructed based on cluster analysis using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) to elucidate the relationships among the wild populations and validate their identities.

The analysis revealed that 95 of the 104 amplified bands were polymorphic, indicating a genetic polymorphism rate of 91.35%. This high level of polymorphism underscores the effectiveness of RAPD markers in assessing genetic diversity and verifying the authenticity of wild populations of Dendrobium officinale. The study demonstrates that RAPD markers are a valuable tool for evaluating genetic variation and ensuring accurate molecular authentication of these populations.

Wang et al. (2009) assessed the genetic diversity and phylogenetic relationships of 31 *Dendrobium* species using 17 Inter-Simple Sequence Repeat (ISSR) primers. The analysis produced 2,368 bands across 278 loci, exhibiting 100% polymorphism at the genus level. ISSR fingerprinting enabled precise differentiation of each species, with species-specific ISSR markers identified in 9 of the 31 species tested. Cluster analysis using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) revealed six distinct clusters, underscoring the polyphyletic nature of the genus. The study demonstrates that ISSR markers are highly effective for evaluating genetic diversity and molecular identification at the species level.

In a study by Asahina et al. (2010), phylogenetic relationships among *Dendrobium* plants were investigated using DNA barcodes comprising two plastid genes: the large subunit ribulose-1,5-bisphosphate carboxylase (rbcL) and maturase-coding gene (matK). The study focused on five medicinal *Dendrobium* species: *D. fimbriatum*, *D. nobile*, *D. moniliforme*, *D. tosaense*, and *D. pulchellum*. Phylogenetic trees constructed from matK sequences effectively differentiated between species, whereas rbcL showed lower species discrimination capability due to limited variation. The combined analysis of matK sequences for *D. officinale* and *D. tosaense* revealed a strong genetic relationship, enhancing our understanding of their taxonomic identities.

Li et al. (2021) explored DNA barcoding for the identification of orchid species within the Orchidaceae family, targeting the chloroplast genome. Four chloroplast genes—matK, rbcL, ycf1, and ndhF—and three combined sequences (matK+ycf1, matK+rbcL, and ndhF+ycf1) were analyzed. Phylogenetic and SNP site analyses established a theoretical framework for species identification, germplasm conservation, and novel applications. The study found that ycf1 and ndhF sequences could identify orchids at both the genus and species levels, with combined sequences (ndhF + ycf1 and matK + ycf1) providing effective identification at these levels. This research represents an initial effort to develop targeted DNA barcodes for orchids, facilitating their identification and conservation through a DNA QR code ID system.

In a study on morphologically similar *Coelogyne* species endemic to Peninsular Malaysia, Kok Hon et al. (2020) selected *C. tiomanensis*, *C. stenochila*, and *C. kaliana* for genetic differentiation. Due to their high morphological similarity, reliable species-specific identification was challenging. The researchers developed RAPD-based SCAR (Sequence Characterized Amplified Region) markers to distinguish among these species. SCAR markers demonstrated the ability to differentiate

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between closely related species based on positive amplifications in targeted samples and varying amplification sizes. Although not all SCAR markers were perfectly specific, they effectively distinguished the three *Coelogyne* species, a feat not achievable with traditional morphological methods.

Kress et al. (2005) proposed that species identification of orchids and other plants would benefit from using multiple genetic markers rather than relying on a single locus. Multi-locus barcoding, which employs several genetic markers, offers greater reliability and accuracy for species resolution.

In 2007, Kress and Erickson achieved an 85% species resolution for angiosperms using a two-locus barcode (trnH-psbA + rbcL), surpassing the previous 82.6% resolution achieved with the single-locus trnH-psbA. Wu et al. (2010) suggested that trnH-psbA combined with trnF-ndhJ could serve as a potential barcode for the *Oncidium* genus, given its ability to resolve the phylogenetic positions of thirteen out of fifteen hybrid varieties.

Kim et al. (2013) proposed that optimal barcoding for Korean orchids could be achieved using a combination of three intergenic spacers: trnH-psbA, atpF-atpH, and psbK-psbI. This combination reached a 98.8% resolution rate across 26 possible combinations of five regions (matK, rbcL, psbK-psbI, atpF-atpH, and trnH-psbA).

Overall, combining single or multi-locus barcodes can

Table 2. Techniques Employed for identification of orchid varieties.

SL.NO.	TECHNIQUES USED	SAMPLES	OBSERVATION	REFERENCE
1	Flower Characterization Leaf Characterization	10 Indonesian native orchids of *Dendrobium* genus	**Differences in Flowering Position and Labellum Characteristics:** Variations in the positioning of flowers and the distinct colors and forms of the labellum (lip) are notable distinguishing features among orchid species. The labellum's diverse hues and morphological forms can be crucial for accurate species identification and classification within the Orchidaceae family. **Variations in Leaf Morphology:** Differences in leaf morphology, including shape, tip structure, surface texture, and arrangement, are significant for species identification. Specific leaf shapes, tip types, surface textures, and the pattern of leaf arrangement can provide valuable diagnostic features for distinguishing between closely related orchid species.	Indraloka et al. (2019)

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2	Flower Characterization Growth Pattern	34 types of orchids belonging to 15 genera in Bengkulu Province	**Variations in Labellum Structure, Color, and Form:** The labellum, or lip, exhibits considerable diversity in structure, coloration, and morphological form among orchid species. These variations can significantly aid in species identification and classification. The labellum's unique attributes, including its shape, size, and color patterns, serve as critical diagnostic features for distinguishing between species within the Orchidaceae family. **Divergences in Perianth Structure (Sepal and Petal):** Differences in the perianth, encompassing both sepals and petals, contribute to the morphological diversity observed in orchids. Variations in the size, shape, and texture of the sepals and petals are essential for accurate species differentiation. These structural differences provide additional key indicators for taxonomic classification and contribute to the overall understanding of orchid floral diversity. **Monopodial Growth Pattern (5.8%):** The monopodial growth pattern, characterized by a single, unbranched stem that continues to grow apically while producing lateral roots, occurs in approximately 5.8% of orchid species. This growth form is distinguished by its vertical, single-stem structure which contrasts with other growth patterns. **Sympodial Growth Pattern (94.12%):** The sympodial growth pattern, which accounts for approximately 94.12% of orchid species, is characterized by a growth habit where the main stem terminates and new growth arises from lateral shoots or pseudobulbs. This pattern often results in a branching or spreading growth form, which is common among many orchid species. The predominance of this growth form highlights its evolutionary advantages and adaptability within the diverse Orchidaceae family.	Miswarti et al. (2021)

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3	DNA Fingerprinting using ISSR Technique	1 *Ascocentrum* 1 *Rhynchostylis* 12 *Dendrobium*	Ten ISSR (Inter-Simple Sequence Repeat) primers were utilized to amplify DNA extracted from selected orchid species. Out of the ten primers, eight successfully generated amplification across all orchid species under study. This outcome underscores the high level of polymorphism inherent in ISSR markers, demonstrating their efficacy in revealing genetic diversity among the orchid species examined.	Pongsrila et al. (2017)
4	Identification using ITS2 DNA Barcode region.	43 *Dendrobium* samples	Ten ISSR (Inter-Simple Sequence Repeat) primers were employed to amplify DNA from various orchid species. Of the ten primers used, eight successfully produced amplifications in all the orchid species analyzed. This result highlights the high polymorphism of ISSR markers, affirming their effectiveness in detecting and elucidating genetic diversity among the studied orchid species.	Feng et al. (2015)
5	Identification using primer sets of *rbcL*, ITS2 and *matK*.	*Vanda tricolor*	Three primer sets—rbcL, ITS2, and matK—were employed for the amplification of isolated DNA. Subsequent sequence alignment and analysis were conducted using BLAST software. The results indicated that the ITS2 primer set demonstrated superior specificity for species identification compared to the other primers. This suggests that ITS2 is particularly effective for accurately distinguishing between species within the studied group.	Su'udi et al. (2022)

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6	DNA Barcoding technique using Single locus and Multi locus barcodes of ITS, ITS2, *matK*, *rbcL*, *trnH-psbA*	Medicinal Orchids	Approximately 7,000 sequences were amplified and analyzed for five distinct barcodes and their seven combinations, encompassing both nuclear and plastid DNA. Among the single-locus barcodes, ITS exhibited the highest efficiency and performance. When considering multi-locus barcodes, the combination of ITS and matK (ITS + matK) provided the most effective species discrimination. This underscores the superior capacity of ITS + matK for distinguishing between species compared to other barcode combinations.	Raskoti et al. (2021)
7	Identification based on partial ribosomal DNA (rDNA) sections containing the ITS 1, 5.8S, and ITS 2 regions.	*Dendrobium tosaense*, *Dendrobium moniliforme*, *Dendrobium officinale*	Approximately 7,000 sequences were amplified and analyzed for five distinct barcodes and their seven combinations, encompassing both nuclear and plastid DNA. Among the single-locus barcodes, ITS exhibited the highest efficiency and performance. When considering multi-locus barcodes, the combination of ITS and matK (ITS + matK) provided the most effective species discrimination. This underscores the superior capacity of ITS + matK for distinguishing between species compared to other barcode combinations.	Cheng et al. (2020)
8	Identification using RAPD (Random Amplified Polymorphic DNA) marker	8 distinct wild populations of *Dendrobium officinale*	It was found that 95 of the 104 amplified bands were polymorphic, representing genetic polymorphism of 91.35 percent. On the basis of cluster analysis by UPGMA the relationships between the wild populations were examined; and all wild populations were verified.	Ding et al. (2005)

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9	Determination of genetic diversity by employing ISSR Fingerprinting technique	*Dendrobium*	A total of 2,368 bands were generated from 278 loci, displaying complete polymorphism at the genus level. Additionally, species-specific ISSR markers were identified in nine out of the 31 Dendrobium species tested. These findings underscore the efficacy of ISSR markers as a robust molecular tool for species identification and classification within the Dendrobium genus.	Wang et al. (2009)
10	DNA Barcoding employing sequences of two plastid genes: *rbcL* and *matK*.	*D. fimbriatum*, *D. nobile*, *D. moniliforme*, *D. tosaense*, *D. pulchellum*	Following the amplification with single-locus barcodes, rbcL and matK, a phylogenetic tree was constructed and analyzed. The results revealed that rbcL exhibited a lower capacity for species discrimination relative to matK as a single-locus barcode. This suggests that matK provides superior resolution for distinguishing between species compared to rbcL.	Asahina et al. (2010)
11	Phylogenetic and SNP site analyses using chloroplast genes - *rbcL*, *matK*, *ndhF*, and *ycf1* in conjunction with three combined sequences— *matK+rbcL*, *matK+ycf1*, and *ndhF+ycf1*	Plants belonging to *Orchidaceae*	Based on genetic distance-based phylogenetic analyses, (using MEGA7.0 sofware based on the Neighbor Joining method and Kimura 2-parameter model), it was demonstrated that *ndhF and ycf1* among single locus barcodes and *matK + ycf1* and *ndhF + ycf1* among multi locus barcodes performed better in Orchid species identification.	Li et al. (2021)

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12	Identification using RAPD-based SCAR (Sequence Characterized Amplified Region) markers	*Coelogyne tiomanensis*, *Coelogyne stenochila*, *Coelogyne kaliana*	Genetic distance-based phylogenetic analyses, conducted using MEGA 7.0 software with the Neighbor-Joining method and Kimura 2-parameter model, demonstrated that single-locus barcodes ndhF and ycf1, as well as multi-locus combinations matK + ycf1 and ndhF + ycf1, exhibited superior performance in Orchid species identification. These findings indicate that both single-locus and multi-locus barcodes, particularly the specified combinations, are effective for accurately distinguishing Orchid species.	Ong et al. (2017)
13	Identification using Multi- locus barcodes regions	*Oncidium* (KoreanOrchid Species)	A potential barcode for the Oncidium genus has been proposed as a combination of trnH-psbA and trnF-ndhJ. Additionally, the use of three intergenic spacers—atpF-atpH, psbK-psbI, and trnH-psbA—has demonstrated a resolution of 98.8% in identifying Korean orchid species. This high resolution underscores the effectiveness of these barcode combinations for accurate species identification within the Oncidium genus and Korean orchids.	Wu et al. (2010) Kim et al. (2013)

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enhance taxonomic resolution for orchid species. Table 2 summarizes the techniques discussed for orchid species identification and a schematic representation is mentioned in figure 2.

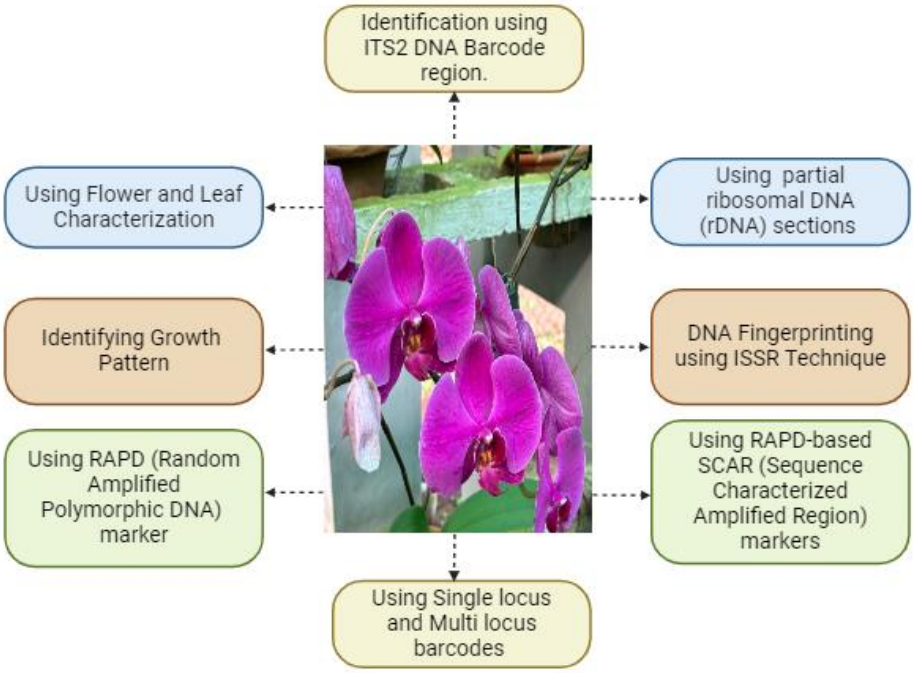

Figure 2. Schematic representation of different identification techniques.

Conservation And Legal Implications

Role in Conservation: Accurate identification of Orchidaceae species plays a pivotal role in conservation efforts. Orchids, known for their ecological, economic, and aesthetic significance, are subject to threats such as habitat destruction, climate change, and illegal trade. Effective conservation strategies hinge on precise species identification, enabling targeted conservation measures, habitat preservation, and restoration efforts (Kumar et al., 2022; Tiwari et al., 2024; Zhang et al., 2022). Key aspects include:

Biodiversity Assessment: Precise identification aids in assessing orchid biodiversity, facilitating the prioritization of species and habitats for conservation efforts. This is crucial for identifying rare, endangered, and endemic species that require immediate attention.

Habitat Protection: Accurate species identification helps delineate critical habitats, promoting the establishment of protected areas and conservation reserves. Understanding species-specific habitat requirements ensures the protection and management of ecological niches vital for orchid survival.

Ex-situ Conservation: Molecular identification techniques are instrumental in ex-situ conservation efforts, such as seed banks and botanical gardens. By accurately cataloging and preserving genetic material, these efforts contribute to the long-term survival of orchid species, especially those facing extinction in the wild.

Reintroduction Programs: Identifying species correctly is essential for successful reintroduction programs. Accurate identification ensures that the right species are reintroduced into suitable habitats, increasing the chances of establishment and survival.

Genetic Diversity Studies: Molecular techniques allow for the assessment of genetic diversity within and between orchid populations. This information is critical for developing conservation strategies that maintain genetic variability, which is essential for species adaptability and resilience.

Community Engagement: Educating local communities about the importance of orchid conservation and the risks of misidentification fosters community involvement in conservation efforts. Engaging communities in monitoring and protecting local orchid populations enhances conservation outcomes.

Legal Framework: The accurate identification of orchid species is integral to the development and enforcement of legal frameworks aimed at preventing illegal trade. Orchids are frequently targeted for their ornamental and medicinal value, leading to rampant illegal collection and trade. Effective legal frameworks depend on robust identification techniques to ensure compliance with international and national regulations (Hinsley et al., 2018; Phelps et al., 2016; Pitogo et al., 2024). Key components include:

International Regulations: Orchid species are listed under the Convention on International Trade in

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Endangered Species of Wild Fauna and Flora (CITES). Accurate species identification is crucial for implementing CITES regulations, which restrict and monitor the international trade of endangered orchid species.

National Legislation: Countries have enacted laws to protect native orchid species from illegal collection and trade. Molecular identification techniques provide reliable evidence for law enforcement agencies to identify and prosecute offenders, thereby strengthening the enforcement of national conservation laws.

Customs and Border Control: Accurate identification techniques enable customs and border control agencies to detect and intercept illegal shipments of orchids. Portable molecular identification devices can be employed at points of entry to verify the identity of traded specimens in real time.

Certification and Permitting: The legal trade of orchids often requires certification and permits. Molecular identification ensures that traded specimens are correctly identified and certified, preventing the laundering of illegal specimens through legal channels.

Traceability and Transparency: Implementing molecular identification in the trade supply chain enhances traceability and transparency. This helps in tracking the origin of orchid specimens, ensuring that they are sourced legally and sustainably.

Judicial Support: Molecular evidence provides robust support in legal proceedings against illegal traders. Courts can rely on scientifically validated identification techniques to make informed decisions, thereby deterring illegal activities through stringent penalties.

Collaboration and Information Sharing: International collaboration and information sharing between countries are essential for combating illegal trade. Establishing databases of molecular markers for orchid species facilitates global efforts in identification and monitoring.

The integration of advanced identification techniques in conservation and legal frameworks is vital for the sustainable management and protection of Orchidaceae species. Accurate identification not only enhances conservation strategies but also strengthens legal measures against illegal trade, ensuring the long-term survival of these ecologically and economically significant plants. Continued research and development of molecular identification methods, coupled with international cooperation, are imperative for the effective conservation of orchid diversity.

Challenges And Limitations

Technical and Practical Challenges: One of the primary technical challenges in Orchidaceae species identification is the complexity and variability of orchid genomes. The high degree of genetic diversity within the family often requires sophisticated and sensitive molecular techniques, which can be resource-intensive and time-consuming. Additionally, the extraction of high-quality DNA from orchids can be difficult due to the presence of secondary metabolites and complex polysaccharides that can inhibit PCR amplification (Ahrens et al., 2017).

Traditional methods of identification, relying heavily on morphological characteristics, can be labor-intensive and require a high level of taxonomic expertise. These methods are often limited by the availability of reproductive structures, such as flowers, which may not be present year-round. Consequently, accurate identification can be hampered by phenotypic plasticity and convergent evolution, where unrelated species develop similar morphological traits in response to analogous environmental pressures (Liu et al., 2023).

In the context of molecular methods, issues such as primer specificity, the potential for contamination, and the requirement for high-throughput sequencing technologies present significant practical barriers. The use of various barcoding markers (e.g., ITS, rbcL, matK) often necessitates the development and optimization of multiple primer sets to ensure broad applicability across diverse orchid species. This can be both costly and technically demanding (Sekse et al., 2017).

Data Limitations and Gaps: The availability and accessibility of comprehensive genetic databases are crucial for the effective use of DNA barcoding and other molecular techniques in orchid identification. However, there are significant gaps in existing databases, with many orchid species either underrepresented or entirely absent. This lack of comprehensive reference sequences limits the reliability of molecular identification and can result in ambiguous or incorrect species assignments. Furthermore, the geographic and ecological diversity of orchids necessitates extensive sampling across different habitats and regions to capture the full range of genetic variation. Current sampling efforts are often biased towards easily accessible areas, leading to a skewed representation of genetic diversity in global databases. This bias can impact the accuracy of phylogenetic studies and conservation efforts (Mahadani et al., 2022).

Data quality is another critical concern. Inconsistent methodologies, varying levels of technical expertise, and differences in data reporting standards can lead to discrepancies and errors in genetic databases. Ensuring the accuracy, consistency, and standardization of data collection and reporting practices is essential for

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advancing orchid species identification (Chen et al., 2020).

Ethical Considerations: Ethical considerations in the identification and conservation of orchid species are multifaceted. The high commercial value and aesthetic appeal of orchids make them targets for illegal collection and trade, contributing to the decline of wild populations and the loss of genetic diversity. Accurate identification is crucial for the enforcement of international regulations such as the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), which seeks to protect endangered species from over-exploitation.

Researchers must balance the need for comprehensive sampling with the potential impact on natural populations. Over-collection for scientific purposes can inadvertently contribute to the depletion of rare or endangered species. Ethical guidelines and permits are necessary to ensure that sampling is conducted responsibly and sustainably (Wang et al., 2024).

The use of advanced molecular techniques also raises ethical concerns related to data ownership and access. Indigenous knowledge and the genetic resources of orchids often originate from biodiverse regions inhabited by indigenous communities. The principles of prior informed consent and benefit-sharing must be adhered to, respecting the rights of these communities and ensuring that they benefit from the scientific and commercial use of their biological resources (Sherkow et al., 2022).

Addressing these challenges and limitations is vital for the continued advancement of orchid species identification. Technological innovations, improved data collection practices, and adherence to ethical standards will enhance the accuracy, efficiency, and sustainability of identification methods, contributing to the conservation and protection of this diverse and valuable plant family.

Recommendations for future research:

Building on the comprehensive review of traditional and molecular methods for Orchidaceae species identification, several avenues for future research are proposed to enhance the accuracy, efficiency, and applicability of these identification techniques:

Development of Multi-Locus Barcodes: While single-locus barcodes have proven effective in certain contexts, the integration of multi-locus barcodes could significantly improve the resolution and accuracy of species identification. Future studies should focus on identifying and validating optimal combinations of genetic markers that can provide a higher level of discrimination across diverse orchid species.

Advancement in Portable Identification Technologies: There is a pressing need for the development of portable, handheld devices capable of in-situ species identification. Incorporating advanced molecular techniques and DNA barcoding into user-friendly, field-deployable tools will facilitate real-time identification of orchid species, aiding in conservation efforts and curbing illegal trade.

Integration of Artificial Intelligence and Deep Learning: The application of artificial intelligence (AI) and deep learning algorithms in species identification holds great promise. Future research should explore the potential of these technologies to analyze large datasets of genetic information, automate species identification, and predict evolutionary relationships with high precision.

Enhancement of Genetic Databases: The accuracy of molecular identification methods is heavily reliant on comprehensive and well-curated genetic databases. Efforts should be directed toward expanding existing databases with high-quality sequences from a broader range of orchid species, ensuring that these databases are accessible and regularly updated.

Exploration of Epigenetic Markers: Epigenetic modifications can provide additional layers of information for species identification. Future research should investigate the potential of epigenetic markers, such as DNA methylation patterns, to complement traditional genetic markers and improve the resolution of species identification.

Application of Next-Generation Sequencing (NGS) Technologies: The utilization of next-generation sequencing technologies can revolutionize orchid species identification by enabling high-throughput analysis of multiple genetic markers simultaneously. Research should focus on optimizing NGS protocols for cost-effective and rapid species identification, making this technology accessible for widespread use.

Interdisciplinary Approaches to Conservation: Combining molecular techniques with ecological, geographical, and phenotypic data can provide a holistic approach to orchid conservation. Future studies should adopt interdisciplinary methodologies to understand the interactions between genetic diversity, environmental factors, and species distribution, thereby informing more effective conservation strategies.

Public Awareness and Policy Advocacy: Raising public awareness about the importance of orchid

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conservation and the threats posed by illegal trade are crucial. Research should also focus on developing policy recommendations and advocacy strategies to strengthen legal frameworks and enforcement mechanisms for the protection of orchid species.

Discussion

A comprehensive survey of the methods used to identify orchid species is provided in this manuscript. Traditional identification is used for identifying orchid species based on morphological and phenotypic characters such as flower and labellum structure, leaf structure, stem and pseudobulb characteristics and root morphology. The lip or labellum, a single petal modified to serve as a landing platform for pollinators, varies in shape and color among orchid species as mentioned by Dodson (Dodson, 2024). It was found that the labellum of *Paphiopedilum rungsuriyanum* is V-shaped and brownish, while Cypripedium reginae has a white, funnel-like labellum with magenta lines (McGough et al., 2004). This variation can be used for identification purposes. Besides floral traits, leaf and stem structures also exhibit species-specific variations crucial for identification. There are some orchids which are monopodial while some are sympodial in nature. In terms of growth, orchids are epiphytic, terrestrial, saprophytic and lithophytic as well. But, consistent identification is challenging due to the limitations of phenotypic plasticity and environmental variability, despite the informative nature of these traits. The manuscript underscores the increasing significance of molecular techniques, including DNA barcoding (utilizing sequences such as ITS, rbcL, and matK), ISSR markers, RAPD, and SCAR markers. These methods are especially beneficial when floral traits are unavailable and enable more precise identification. DNA barcoding is recognized as a potent approach to the genetic identification of species, particularly orchids that are unlawfully traded at juvenile or sterile stages, which complicates the process of morphological identification. Although traditional approaches are helpful for species identification, they require integration with molecular approaches as well for better accuracy. Illegal trade in orchids poses a significant threat to biodiversity, this manuscript examines the necessity of both traditional and modern molecular techniques for accurate identification in order to mitigate the substantial threat to biodiversity posed by the illegal trade in orchids and for its conservation. Now-a-days, ecological or environmental DNA (eDNA) is also an emerging technique employed for plant species identification. It offers an efficient analysis of both visible and invisible biodiversity with a higher resolution than traditional methods can provide. By analysing the DNA present in a soil sample, it is possible to get a better estimate of the diversity in the environment than in a traditional field inventory, as many species can be difficult to find or classify based on morphological characters. According to Banerjee et al. (2022), despite only 13% of eDNA studies focusing on plants, there is significant potential for its use in detecting invasive, endangered, and rare species, as well as studying community interactions and responses to anthropogenic pressures. This technique can be applied to orchid plants, too. This study finds that eDNA methods are frequently as effective, if not more so, than traditional methods. However, the combination of both methods improves the detection of species (Banerjee et al., 2022).

Apart from these, advancements in statistical approaches and techniques like *Bayesian inference* or Machine Learning and Deep Learning can also be used for accurate species identification, although these techniques are not included in this study. As described by Apriyanti et al.(2024) the significance of explicit, botanical descriptions as prominent characteristics that are obtained from the taxonomic characteristics of plants, as employed in conventional plant identification and then combining this method with the advanced technology of 'Deep Neural Network' in order to develop a completely new automated identification technique. The research concentrates on orchid-specific characteristics, including the number of flowers, inflorescence, labellum texture, and flower hue. These features are extracted using deep neural networks and subsequently employed for species classification using algorithms such as Naive Bayes and Tree-Augmented Bayesian Networks (TAN). The system achieved an accuracy of 88.9% while justifying the obtained classification and identification of orchid plants. This newly developed automated identification method utilizes the potentialities of the Deep neural network architectures for flower image interpretation and clear explanation of the resulted plant identification based on taxonomic characteristics (Apriyanti et al. 2024) as described in figure 3.

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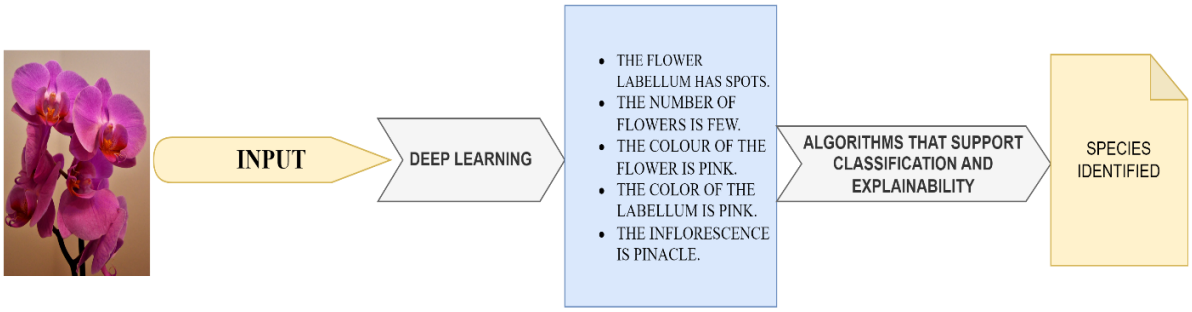

Figure 3. Allignment of 'Deep Neural Network' with Taxonomic characteristics for orchid species identification.

Conclusion

Accurate identification of Orchidaceae species is essential for effective conservation efforts, aiding in biodiversity assessment, habitat protection, ex-situ conservation, reintroduction programs, and genetic diversity studies. Precise identification allows for the prioritization of rare and endangered species, the establishment of protected areas, and successful reintroduction of species into suitable habitats. Molecular techniques support ex-situ conservation by preserving genetic material in seed banks and botanical gardens, and they facilitate community engagement by educating local populations on the importance of orchid conservation.

In the legal context, robust identification techniques are critical for enforcing regulations and preventing illegal trade. Accurate identification ensures compliance with international agreements such as CITES, and supports national legislation aimed at protecting native species. It enables customs and border control agencies to detect and intercept illegal shipments, enhances traceability and transparency in the trade supply chain, and provides strong evidence in legal proceedings against offenders. Collaboration and information sharing on molecular markers among countries are essential for global monitoring and enforcement.

Integrating advanced identification methods into conservation and legal frameworks is vital for the sustainable management of Orchidaceae species. This approach not only strengthens conservation strategies but also bolsters legal measures against illegal trade, ensuring the long-term survival of these ecologically and economically significant plants. Continued research and international cooperation are imperative for the effective conservation of orchid diversity.

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Conflict of Interest

The authors declare no conflict of interest.

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