

DNA Barcoding in Euphorbia Species: Current Status, Challenges, and Future Perspectives

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ABSTRACT

The genus *Euphorbia* L., one of the largest genera within the family Euphorbiaceae, comprises approximately 2,000–2,200 species distributed across diverse ecological regions worldwide. Owing to its extensive morphological diversity, phenotypic plasticity, convergent evolution, and complex taxonomic relationships, accurate species identification within the genus remains a significant challenge. Traditional morphological approaches are often insufficient for distinguishing closely related species, particularly when diagnostic characters are absent or highly variable. In this context, DNA barcoding has emerged as a powerful molecular tool for species identification, taxonomic clarification, biodiversity assessment, and conservation research. This review provides a comprehensive overview of DNA barcoding applications in *Euphorbia*, with emphasis on the major barcode markers used in plant identification, including chloroplast loci (*rbcL*, *matK*, *trnH-psbA*, and *ycf1*) and nuclear markers (ITS and ITS2). The principles, methodology, and effectiveness of these markers are discussed, along with their roles in species discrimination, detection of cryptic species, authentication of medicinal plants, phylogenetic reconstruction, and biodiversity conservation. Comparative evaluation of barcode markers indicates that while *rbcL* and *matK* remain the standard core plant barcodes, nuclear ITS regions generally provide higher species-level resolution. Emerging markers such as *ycf1* and multilocus barcode combinations have demonstrated improved discriminatory power in taxonomically complex groups.

Despite significant progress, several challenges continue to limit the effectiveness of DNA barcoding in *Euphorbia*, including low genetic divergence among closely related species, hybridization, polyploidy, incomplete reference databases, and technical constraints associated with molecular analyses. Recent advances in DNA metabarcoding, genome skimming, chloroplast genome sequencing, next-generation sequencing technologies, and integrative taxonomy offer promising solutions to these limitations and are expected to enhance future taxonomic and evolutionary studies. Overall, DNA barcoding has substantially improved species identification and systematic research in *Euphorbia*. Continued development of comprehensive reference databases, adoption of multilocus and genomic approaches, and integration of molecular and morphological data will further strengthen taxonomic resolution and contribute to the conservation and sustainable utilization of *Euphorbia* diversity worldwide.

Keywords: *Euphorbia*, DNA barcoding, *rbcL*, *matK*, ITS, species identification, molecular taxonomy, phylogenetics, biodiversity conservation, Euphorbiaceae.

1. Introduction

Species identification is fundamental to biodiversity assessment, conservation planning, ecological studies, and sustainable resource use. Traditionally, plant taxonomy has relied on morphological traits such as vegetative and reproductive characters for species delimitation. However, morphology-based identification is often limited by phenotypic plasticity, developmental stage, environmental variation, and the absence of diagnostic features in incomplete specimens (Drew, 2011). These constraints are especially evident in damaged, sterile, or juvenile plant materials.

Advances in molecular biology have transformed taxonomy by introducing DNA-based identification tools that complement classical morphology. Molecular approaches improve species delimitation and help resolve evolutionary relationships, particularly in taxonomically complex groups where morphological convergence obscures true relationships (Hebert et al., 2003).

DNA barcoding is a standardized molecular technique that uses short, conserved DNA regions to identify species. First proposed by Hebert et al. (2003), it provides rapid and reliable species recognition across diverse biological groups. Barcode sequences are selected based on their ability to show low intraspecific variation and high interspecific divergence. This method enables species identification regardless of life stage, specimen condition, or morphological completeness (Hebert et al., 2003; Kress & Erickson, 2007). Global initiatives such as CBOL and BOLD have standardized protocols and expanded access to reference sequence databases, enhancing the global applicability of DNA barcoding (CBOL Plant Working Group, 2009).

In plants, mitochondrial DNA evolves too slowly for species discrimination; therefore, chloroplast and nuclear regions are used instead. Common plant barcode markers include chloroplast genes *rbcL* and *matK*, the intergenic spacer *trnH-psbA*, and nuclear ITS regions (Kress & Erickson, 2007; Hollingsworth et al., 2011). Among these, *rbcL* and *matK* are recognized as core plant barcodes due to their universality and discriminatory power, while ITS provides higher resolution at the species level (CBOL Plant Working Group, 2009; China Plant BOL Group, 2011). Multilocus barcoding approaches are often required because no single locus is sufficient across all plant groups (Hollingsworth et al., 2011).

The family Euphorbiaceae, with approximately 8,900 species across more than 300 genera, is one of the largest angiosperm families (Govaerts et al., 2000). Within it, the genus *Euphorbia* is exceptionally diverse, containing about 2,000–2,200 species distributed globally (Horn et al., 2012). *Euphorbia* species occupy a wide range of habitats and exhibit remarkable morphological variation, including herbs, shrubs, succulents, and trees. The genus is characterized by the unique cyathium inflorescence, a key taxonomic feature (Bruyns et al., 2006).

Euphorbia is economically important due to its medicinal, ornamental, and ecological value. Many species are used in traditional medicine, while others are cultivated as ornamentals or sources of bioactive compounds (Mwine & Van Damme, 2011). However, taxonomic identification is complicated by morphological convergence, high species richness, and complex evolutionary history. Molecular phylogenetic studies have supported its division into four subgenera: *Esula*, *Euphorbia*, *Chamaesyce*, and *Athymalus* (Horn et al., 2012; Yang et al., 2012).

Given its complexity and economic importance, *Euphorbia* is an ideal model for evaluating DNA barcoding in plant systematics. This review therefore examines barcode principles, marker systems, *Euphorbia* taxonomy, applications, limitations, and future directions in molecular identification and biodiversity research (Hollingsworth et al., 2011; Horn et al., 2012).

2. DNA Barcoding: Principles and Methodology

2.1 Historical Development of DNA Barcoding

DNA barcoding is a molecular technique that uses short, standardized DNA sequences to identify and classify organisms. The concept was first proposed by Hebert et al. (2003), who recommended the mitochondrial cytochrome c oxidase I (COI) gene as a universal barcode for animal identification. This approach revolutionized taxonomy by providing a rapid, reliable, and standardized method for species discrimination (Hebert et al., 2003). Since then, DNA barcoding has become a key tool in biodiversity research, species discovery, and taxonomic studies (Vohra & Khera, 2013; Braukmann et al., 2019).

However, COI is not suitable for plants due to the slow evolutionary rate of plant mitochondrial DNA. As a result, plant barcoding shifted toward chloroplast and nuclear markers. The Consortium for the Barcode of Life (CBOL) Plant Working Group recommended *rbcL* and *matK* as core plant barcode regions (CBOL Plant Working Group, 2009; Hollingsworth et al., 2011). Later studies identified additional useful markers such as ITS, ITS2, and *trnH-psbA* for improving species-level resolution (Kress & Erickson, 2007; Chen et al., 2010; Hollingsworth et al., 2016).

International initiatives such as the Barcode of Life Data System (BOLD) and the International Barcode of Life Project (iBOL) have further advanced the field by providing standardized protocols and global reference databases (Ratnasingham & Hebert, 2007). Continuous technological improvements and global collaboration have strengthened the role of DNA barcoding in modern taxonomy (Grant et al., 2021).

2.2 Characteristics of an Ideal DNA Barcode

An ideal DNA barcode should show high interspecific variation and low intraspecific variation to ensure reliable species discrimination (Hollingsworth et al., 2011). It should also contain conserved regions for universal primer design and successful amplification across diverse taxa (Kress & Erickson, 2007).

Additional requirements include high PCR success, easy sequencing and alignment, reproducibility across laboratories, and strong representation in reference databases (Hollingsworth et al., 2016). A good barcode should also be cost-effective and applicable across broad taxonomic groups (Fazekas et al., 2008; Saddhe & Kumar, 2018). Since no single plant marker satisfies all these criteria, multilocus barcoding using combinations of *rbcL*, *matK*, ITS, and *trnH-psbA* is widely adopted (Hollingsworth et al., 2011; Kress & Erickson, 2007).

2.3 Workflow of DNA Barcoding

DNA barcoding follows a standardized workflow involving sampling, DNA extraction, amplification, sequencing, and data analysis (Kress & Erickson, 2012).

2.3.1 Sample Collection

The process begins with collecting plant material, including fresh tissues, herbarium specimens, processed products, or environmental samples. Proper documentation and voucher preservation are essential for accurate identification and reproducibility (Kress & Erickson, 2012).

2.3.2 DNA Extraction

Genomic DNA is extracted using methods such as the CTAB protocol developed by Doyle and Doyle (1987). This method efficiently removes polysaccharides and secondary metabolites common in plants. High-quality DNA is essential for successful downstream amplification, especially in degraded or processed samples (Chen et al., 2010).

2.3.3 PCR Amplification

PCR is used to amplify selected barcode regions such as *rbcL*, *matK*, ITS, ITS2, and *trnH-psbA* (Kress & Erickson, 2007; Chen et al., 2010). The success of amplification depends on DNA quality, primer specificity, and optimized reaction conditions (Kress & Erickson, 2012).

2.3.4 Sequencing

Amplified products are sequenced using Sanger sequencing, which remains the standard due to its accuracy. However, next-generation sequencing (NGS) technologies now enable high-throughput barcoding and environmental DNA analysis, improving efficiency and reducing costs (Shokralla et al., 2014; Grant et al., 2021).

2.3.5 Data Analysis and Species Identification

Sequences are edited, aligned, and compared with reference databases such as GenBank and BOLD (Ratnasingham & Hebert, 2007; Clark et al., 2016). Alignment tools like MAFFT are commonly used for sequence comparison (Kato et al., 2002).

Species identification is based on sequence similarity and the barcode gap, where interspecific variation exceeds intraspecific variation (Puillandre et al., 2012). Phylogenetic and genetic distance analyses further improve species delimitation accuracy and support biodiversity assessment and detection of cryptic species (Hollingsworth et al., 2016; Costion et al., 2011).

3. DNA Barcode Markers Used in Plants

DNA barcoding in plants relies mainly on chloroplast and nuclear genome regions because plant mitochondrial DNA evolves too slowly to provide sufficient species-level variation. An ideal plant barcode should have high universality, reliable amplification, and enough sequence variability to distinguish species effectively (Hollingsworth et al., 2011; Li et al., 2015). Therefore, several chloroplast and nuclear markers have been evaluated for plant identification, phylogenetics, and biodiversity studies.

3.1 Chloroplast Markers

The chloroplast genome is the most commonly used source for plant DNA barcoding due to its conserved structure, uniparental inheritance, and moderate evolutionary rate. Chloroplast markers generally show high amplification success and wide taxonomic applicability (Kress & Erickson, 2007; Li et al., 2015).

3.1.1 *rbcL*

The *rbcL* gene encodes the large subunit of RuBisCO, an essential enzyme in photosynthesis. It is one of the earliest and most widely used plant barcode genes due to its highly conserved primer regions, which allow universal amplification (Kress & Erickson, 2007; Fazekas et al., 2008). Its main strengths are reliability, ease of sequencing, and broad applicability. However, its low sequence variation limits species-level resolution, making it more suitable for higher taxonomic identification. Therefore, it is often combined with other markers such as *matK* and ITS (Hollingsworth et al., 2011; Li et al., 2015).

3.1.2 *matK*

The *matK* gene is one of the fastest-evolving chloroplast coding regions and provides higher discriminatory power than *rbcL* (CBOL Plant Working Group, 2009; Saddhe & Kumar, 2018). It is effective in distinguishing closely related species, although amplification can sometimes be difficult due to primer mismatches. Despite this limitation, the *rbcL* + *matK* combination is widely accepted as a standard plant barcode system.

3.1.3 trnH-psbA

The *trnH-psbA* intergenic spacer is a non-coding chloroplast region with relatively high sequence variability, making it useful for species identification (Kress & Erickson, 2007). It is short and easy to amplify, but alignment issues due to insertions, deletions, and inversions can complicate analysis. Hence, it is usually used as a supplementary marker (Hollingsworth et al., 2011).

3.1.4 ycf1

The *ycf1* gene is one of the most variable chloroplast regions and has emerged as a promising next-generation barcode. It provides high species discrimination and often outperforms traditional markers like *rbcL* and *matK* (Dong et al., 2015; Li et al., 2015).

3.2 Nuclear Markers

Nuclear markers evolve faster than chloroplast regions and often provide higher species-level resolution (Hollingsworth et al., 2016).

3.2.1 ITS

The ITS region, located between ribosomal RNA genes, is one of the most widely used nuclear barcodes due to its high variability and strong discriminatory power (China Plant BOL Group, 2011). It is highly effective for distinguishing closely related species but may face issues such as paralogy, fungal contamination, and incomplete concerted evolution (Hollingsworth et al., 2011).

3.2.2 ITS2

ITS2 is a shorter subregion of ITS that is easier to amplify from degraded or processed samples. It has shown high success rates in medicinal plant identification and biodiversity studies, making it valuable for applied taxonomy and conservation (Chen et al., 2010; Hollingsworth et al., 2016).

3.3 Comparison of Common Plant DNA Barcodes

Different DNA barcode markers exhibit distinct strengths and limitations. Consequently, no single marker is universally effective across all plant groups. Multilocus approaches generally provide the highest levels of species discrimination and taxonomic accuracy (Fazekas et al., 2008; Hollingsworth et al., 2011).

Marker	Genome	Advantages	Limitations
<i>rbcL</i>	Chloroplast	High universality, easy amplification, excellent sequence quality	Low species-level resolution
<i>matK</i>	Chloroplast	High discriminatory power, recommended core barcode	Amplification difficulties in some taxa
<i>trnH-psbA</i>	Chloroplast	High sequence variation, useful supplementary marker	Alignment challenges due to indels and inversions
<i>ycf1</i>	Chloroplast	Very high variability and species discrimination	Limited universal primer availability
ITS	Nuclear	Excellent species resolution, high variability	Potential paralogy and contamination issues
ITS2	Nuclear	Short length, suitable for degraded samples, high identification success	Less phylogenetic information than full ITS

Overall, the combined use of chloroplast markers (*rbcL* and *matK*) with nuclear regions such as ITS or ITS2 often provides the most reliable framework for plant species identification. Recent evidence also suggests that *ycf1* may become an important component of future plant DNA barcoding systems because of its exceptional discriminatory power (Dong et al., 2015; Hollingsworth et al., 2016; Li et al., 2015).

4. The Genus *Euphorbia*: Taxonomy and Significance

4.1 Taxonomic Position

Euphorbia is one of the largest and most taxonomically complex genera of angiosperms, belonging to the family Euphorbiaceae within the order Malpighiales. Euphorbiaceae comprises approximately 300 genera and more than 8,900 species distributed throughout tropical, subtropical, and temperate regions of the world. Within this family, *Euphorbia* is the largest genus, encompassing nearly 2,000–2,200 species and representing a major component of global plant diversity (Steinmann & Porter, 2002; Frodin, 2004).

A defining characteristic of the genus is the cyathium, a unique inflorescence composed of highly reduced unisexual flowers arranged in a structure that resembles a single flower. This specialized reproductive feature is a key diagnostic trait for species identification and classification. Molecular phylogenetic studies have confirmed the monophyly of *Euphorbia* and supported its division into four major subgenera: *Esula*, *Euphorbia*, *Chamaesyce*, and *Athymalus* (Steinmann & Porter, 2002; Horn et al., 2012). In addition, phylogenetic evidence has shown that several previously recognized genera are nested within *Euphorbia*, leading to a broader and more inclusive circumscription of the genus (Bruyns et al., 2006; Yang et al., 2012).

4.2 Diversity and Distribution

The genus *Euphorbia* exhibits exceptional morphological, ecological, and geographical diversity, making it one of the most widely distributed plant groups in the world. Species occur in diverse habitats, including deserts, grasslands, forests, wetlands, coastal regions, and mountainous ecosystems. Although nearly cosmopolitan—present on all continents except Antarctica—the highest species richness occurs in Africa, Madagascar, Asia, and the Americas (Bruyns et al., 2006).

Members of *Euphorbia* display remarkable variation in growth forms, ranging from annual and perennial herbs to shrubs, climbers, succulents, and tree-like species. Many succulent taxa have independently evolved cactus-like morphologies as adaptations to arid environments, representing a strong example of convergent evolution in plants. Molecular studies suggest that repeated adaptation to drought-prone habitats has driven the emergence of succulent forms across multiple evolutionary lineages within the genus (Horn et al., 2012).

The ecological adaptability of *Euphorbia* is further reflected in its wide range of physiological and morphological strategies. Desert-adapted species often possess reduced leaves, water-storing stems, and specialized photosynthetic pathways, while other species thrive in tropical forests and temperate ecosystems. This ecological versatility has enabled the genus to occupy a broad spectrum of environmental conditions and contribute significantly to global plant diversity.

4.3 Economic Importance

Species of *Euphorbia* have considerable economic value in horticulture, landscaping, traditional medicine, and industrial applications. Many species are cultivated as ornamentals due to their unique plant architecture, colorful bracts, and adaptability to diverse environments. One of the most economically significant species is *Euphorbia pulcherrima* (poinsettia), widely grown and traded globally during the Christmas season.

In addition, several species are valued in xeriscaping due to their drought tolerance and low maintenance requirements. The genus produces a characteristic latex rich in secondary metabolites, including diterpenoids, triterpenoids, flavonoids, and phenolic compounds, many of which show promising pharmacological and industrial potential (Mwine & Van Damme, 2011). These properties have increased scientific and commercial interest in the genus as a source of novel natural products.

4.4 Medicinal Importance

The medicinal importance of *Euphorbia* has been recognized in traditional medicine systems across Africa, Asia, Europe, and the Americas. Species are used to treat skin disorders, inflammatory conditions, respiratory ailments, gastrointestinal diseases, infections, and parasitic infestations (Mwine & Van Damme, 2011).

Phytochemical studies have identified diverse bioactive compounds such as diterpenes, triterpenes, flavonoids, alkaloids, tannins, and phenolics, which are associated with antimicrobial, antiviral, antioxidant, anti-inflammatory, and anticancer activities (Khan et al., 2020). However, some species produce toxic latex capable of causing irritation and poisoning, making accurate species identification essential for safe medicinal use. DNA barcoding has therefore become an important tool for authenticating medicinal *Euphorbia* species and ensuring quality control in herbal products.

4.5 Taxonomic Challenges in *Euphorbia*

Despite advances in systematics, *Euphorbia* remains taxonomically challenging due to high species diversity and complex evolutionary processes. Morphological plasticity often leads to variation in vegetative and reproductive traits influenced by environmental conditions, complicating species delimitation (Steinmann & Porter, 2002).

Convergent evolution, especially among succulent species, results in unrelated taxa exhibiting similar morphological traits. Hybridization, polyploidy, and rapid speciation further obscure species boundaries and complicate classification. Additionally, repeated radiations and geographically isolated populations have generated complex phylogenetic patterns that are difficult to resolve using morphology alone (Yang et al., 2012; Horn et al., 2012).

As a result, molecular approaches such as DNA barcoding and phylogenetic analysis are increasingly essential for resolving taxonomic uncertainties. The integration of molecular and morphological data is now considered the most reliable strategy for achieving accurate species identification and improving taxonomic resolution within this highly diverse genus.

5. Applications of DNA Barcoding in *Euphorbia*

DNA barcoding has emerged as one of the most significant molecular tools in modern plant systematics, providing rapid, accurate, and standardized approaches for species identification and biodiversity assessment. In the genus *Euphorbia*, which comprises approximately 2,000–2,200 species exhibiting considerable morphological diversity and taxonomic complexity, DNA barcoding has become increasingly important for addressing challenges in species delimitation, medicinal plant authentication, phylogenetic reconstruction, and conservation planning. Barcode markers such as *rbcL*, *matK*, ITS, ITS2, and *trnH-psbA* have significantly improved species discrimination and complemented traditional morphological taxonomy (Kress & Erickson, 2007; Hollingsworth et al., 2011; Hollingsworth et al., 2016).

5.1 Species Identification

Species identification is the primary application of DNA barcoding in *Euphorbia*. Morphological identification is often difficult due to phenotypic plasticity, environmental variation, and convergent evolution, which result in overlapping diagnostic characters among closely related species (Steinmann & Porter, 2002; Horn et al., 2012). DNA barcoding provides an objective and reproducible system based on genetic variation.

Chloroplast markers (*rbcL*, *matK*) and nuclear markers (ITS, ITS2) are widely used in species identification, with multilocus combinations providing higher resolution than single markers due to complementary genomic information (CBOL Plant Working Group, 2009; China Plant BOL Group, 2011; Hollingsworth et al., 2011). In *Euphorbia*, barcoding is particularly valuable for identifying sterile, juvenile, or fragmented specimens and herbarium materials where morphological traits are insufficient.

5.2 Detection of Cryptic Species

DNA barcoding is essential for detecting cryptic species, which are genetically distinct but morphologically similar lineages. In *Euphorbia*, convergent evolution often produces nearly identical morphologies in unrelated lineages, masking true diversity (Hebert et al., 2003). Molecular identification helps reveal hidden evolutionary lineages and improves biodiversity estimates.

This has major implications for conservation biology, as cryptic species may represent distinct evolutionary units requiring separate protection strategies (Hollingsworth et al., 2016).

5.3 Authentication of Medicinal Species

Many *Euphorbia* species are widely used in traditional medicine due to antimicrobial, anti-inflammatory, antiviral, antioxidant, and anticancer properties (Mwine & Van Damme, 2011; Khan et al., 2020). However, misidentification and adulteration in herbal markets pose serious risks.

DNA barcoding provides a reliable method for authenticating medicinal plants, particularly in processed products where morphology is absent (Chen et al., 2010). ITS and ITS2 markers are especially effective for identifying medicinal species and detecting adulterants (Chen et al., 2010; China Plant BOL Group, 2011). This is crucial because several *Euphorbia* species contain toxic latex, making accurate identification essential for safety and regulatory control.

5.4 Phylogenetic and Evolutionary Studies

DNA barcoding also contributes to phylogenetic reconstruction and evolutionary analysis. Barcode sequences help infer evolutionary relationships, diversification patterns, and lineage divergence (Hollingsworth et al., 2016).

Molecular evidence supports four major subgenera in *Euphorbia*: *Esula*, *Euphorbia*, *Chamaesyce*, and *Athymalus* (Horn et al., 2012). Barcode data further assist in studying hybridization, polyploidy, adaptive radiation, and convergent evolution. Additionally, these data contribute to biogeographic studies by revealing historical dispersal and diversification patterns.

5.5 Conservation and Biodiversity Assessment

DNA barcoding plays a crucial role in biodiversity conservation and ecological monitoring. Accurate species identification is essential for conservation planning, particularly in species-rich genera like *Euphorbia* (Ratnasingham & Hebert, 2007).

Barcode reference libraries enable identification of rare, endemic, and threatened species from field samples, herbarium specimens, and environmental DNA (Ratnasingham & Hebert, 2007; Hollingsworth et al., 2016). It also supports biodiversity inventories, conservation prioritization, and monitoring of illegal trade in medicinal and ornamental species. Thus, DNA barcoding is increasingly important in global conservation strategies for *Euphorbia* diversity.

6. DNA Barcoding Studies in *Euphorbia*: A Global Overview

DNA barcoding has become an essential molecular approach for species identification, phylogenetic reconstruction, biodiversity assessment, and medicinal plant authentication in *Euphorbia*. As one of the largest and most complex genera of flowering plants, *Euphorbia* presents major taxonomic challenges due to morphological convergence, phenotypic plasticity, and complex evolutionary history. Over time, research has progressed from single-locus chloroplast markers to multilocus barcoding systems, plastome sequencing, and phylogenomic approaches, significantly improving taxonomic resolution (Steinmann & Porter, 2002; Horn et al., 2012; Hollingsworth et al., 2016).

6.1 Early Studies

Early molecular studies in *Euphorbia* focused on resolving phylogenetic relationships and testing morphology-based classifications. These studies revealed that many morphological traits were the result of convergent evolution and did not reflect true evolutionary history (Steinmann & Porter, 2002).

Following the introduction of DNA barcoding by Hebert et al. (2003), chloroplast markers such as *rbcL* and *matK* were adopted for plant identification due to their universality and amplification success (Kress & Erickson, 2007). However, early studies showed limited resolution for closely related species due to low genetic variation (CBOL Plant Working Group, 2009). This highlighted the need for more variable molecular markers (Hollingsworth et al., 2011).

6.2 Recent Advances

Recent advances in sequencing technologies and bioinformatics have expanded DNA barcoding applications in *Euphorbia*. Multilocus approaches combining chloroplast and nuclear markers have improved species resolution (Hollingsworth et al., 2011).

Nuclear markers (ITS, ITS2) provide higher variability and better species discrimination than chloroplast loci (China Plant BOL Group, 2011; Chen et al., 2010). Next-generation sequencing (NGS) technologies have enabled genome skimming and plastome sequencing, allowing access to highly informative markers such as *ycf1*, which often outperform traditional barcodes (Dong et al., 2015).

Phylogenomic studies have clarified evolutionary relationships and supported major subgeneric classifications (Horn et al., 2012). Beyond taxonomy, DNA barcoding is widely applied in medicinal authentication, conservation, and biodiversity monitoring (Mwine & Van Damme, 2011; Khan et al., 2020).

6.3 Marker Performance in *Euphorbia*

6.3.1 *rbcL*-Based Studies

rbcL is widely used due to its universality and high amplification success, making it suitable for large-scale studies (Kress & Erickson, 2007). However, its low variability limits species-level resolution, requiring combination with other markers (CBOL Plant Working Group, 2009; Hollingsworth et al., 2011; Li et al., 2015).

6.3.2 *matK*-Based Studies

matK provides higher resolution due to its faster evolutionary rate and is a core plant barcode marker (CBOL Plant Working Group, 2009). In *Euphorbia*, it is effective for species discrimination but may face amplification challenges (Hollingsworth et al., 2011; Saddhe & Kumar, 2018).

6.3.3 ITS-Based Studies

ITS is one of the most powerful barcode markers due to its high variability and strong discriminatory power (China Plant BOL Group, 2011). In *Euphorbia*, it reveals variation not detected by chloroplast markers (Horn et al., 2012). However, issues such as paralogy, incomplete concerted evolution, and fungal contamination may complicate analysis (Hollingsworth et al., 2011; China Plant BOL Group, 2011).

6.3.4 Multilocus Approaches

No single barcode is sufficient for *Euphorbia*. Therefore, multilocus combinations such as *rbcL* + *matK*, *matK* + *ITS*, and *rbcL* + *matK* + *ITS* are recommended for improved accuracy (Hollingsworth et al., 2011). Integrating highly variable regions such as *ycf1* and using genome skimming and phylogenomics further enhances resolution (Dong et al., 2015; Hollingsworth et al., 2016).

Study	Marker(s) Used	Species/Group Studied	Major Findings
Kress & Erickson (2007)	<i>rbcL</i> , <i>trnH-psbA</i>	Land plants	Improved plant species discrimination using combined markers
China Plant BOL Group (2011)	ITS	Diverse plant taxa	ITS showed high species identification success
Dong et al. (2015)	<i>ycf1</i>	Multiple plant groups	<i>ycf1</i> demonstrated superior discriminatory power
Horn et al. (2012)	Multiple loci	<i>Euphorbia</i> phylogeny	Clarified subgeneric relationships within <i>Euphorbia</i>

7. Comparative Evaluation of DNA Barcode Markers in *Euphorbia*

The efficiency of DNA barcoding in *Euphorbia* is strongly influenced by the choice of genetic markers, as the genus exhibits high species richness, morphological convergence, hybridization, and recent diversification. Because of these factors, no single DNA barcode region provides complete species resolution across all taxa. Consequently, chloroplast markers such as *rbcL*, *matK*, *trnH-psbA*, and *ycf1*, along with nuclear markers including ITS and ITS2, have been extensively evaluated to determine their relative performance in species identification and phylogenetic analysis (Kress & Erickson, 2007; Hollingsworth et al., 2011; Li et al., 2015).

7.1 Species Resolution Efficiency

Species resolution refers to the ability of a marker to distinguish closely related species. Among commonly used loci, the nuclear ITS region generally provides the highest resolution due to its rapid evolutionary rate and high sequence variability (China Plant BOL Group, 2011). It is particularly effective in resolving cryptic and closely related species that cannot be distinguished using plastid markers alone (Hollingsworth et al., 2011).

In contrast, *rbcL* exhibits low interspecific variation, limiting its effectiveness at the species level, although it remains highly reliable for higher-level classification and broad phylogenetic placement (CBOL Plant Working Group, 2009). The *matK* gene offers improved discriminatory power compared to *rbcL* because of its faster

substitution rate, making it useful for species-level identification in many plant groups, including *Euphorbia* (Kress & Erickson, 2007). However, its performance may vary due to amplification difficulties in some taxa.

Barcode Combination	Application	Effectiveness
<i>rbcL</i> + <i>matK</i>	General plant identification	Moderate to high
<i>rbcL</i> + ITS	Species-level discrimination	High
<i>matK</i> + ITS	Closely related species	Very high
<i>rbcL</i> + <i>matK</i> + ITS	Comprehensive taxonomic studies	Excellent
<i>trnH-psbA</i> + ITS	Difficult species complexes	High
<i>ycfI</i> + ITS	Advanced phylogenetic and barcoding studies	Very high

The intergenic spacer *trnH-psbA* provides relatively high variability and can enhance species discrimination when combined with coding regions, although structural variation and alignment complexity may reduce analytical reliability (Kress & Erickson, 2007). Among newer markers, *ycfI* has emerged as one of the most promising chloroplast regions due to its exceptionally high nucleotide variability and strong phylogenetic signal, often outperforming traditional barcode loci in species discrimination (Dong et al., 2015). Overall, ITS and *ycfI* generally show the highest species resolution, whereas *rbcL* provides the lowest but most stable framework for broader comparisons.

7.2 Amplification Success Rate

Amplification success is a critical factor for practical barcoding applications. The *rbcL* gene consistently demonstrates the highest PCR success due to conserved primer-binding regions and universal primer applicability (CBOL Plant Working Group, 2009). In contrast, *matK* often requires taxon-specific or optimized primers because of sequence variation at primer sites, reducing its universality despite its high resolution potential (Hollingsworth et al., 2011).

The *trnH-psbA* spacer generally amplifies well, even from degraded DNA, making it useful for herbarium and processed samples; however, its structural variation can complicate sequence alignment (Kress & Erickson, 2007). Nuclear ITS and ITS2 markers also show relatively high amplification success but may be affected by issues such as fungal contamination and multiple rDNA copies (Chen et al., 2010). Despite these challenges, ITS remains one of the most widely used high-resolution markers in plant barcoding studies.

7.3 Advantages and Limitations of Individual Markers

Each barcode locus presents distinct advantages and limitations. The *rbcL* gene is highly universal, easy to amplify, and well represented in reference databases, making it suitable for preliminary identification and large-scale biodiversity studies. However, its low variability limits species-level resolution (Hollingsworth et al., 2011).

The *matK* gene provides better resolution but suffers from amplification inconsistencies across taxa (CBOL Plant Working Group, 2009). The *trnH-psbA* region is highly variable and useful for discrimination but is often complicated by indels and inversions. The nuclear ITS region offers strong discriminatory power but may be affected by paralogy, incomplete concerted evolution, and contamination issues (Hollingsworth et al., 2011). ITS2, while shorter and more suitable for degraded DNA, provides less phylogenetic information than full ITS sequences (Chen et al., 2010). The *ycfI* gene shows high potential as a next-generation barcode but currently lacks standardized universal primers and comprehensive reference coverage.

7.4 Recommended Barcode Combinations

Because no single locus is sufficient for all *Euphorbia* species, multilocus barcoding remains the most reliable approach. The standard combination *rbcL* + *matK* provides a balance between universality and discriminatory power and is widely recommended for plant barcoding studies (CBOL Plant Working Group, 2009). However, adding ITS significantly improves resolution in taxonomically complex groups such as *Euphorbia* (China Plant BOL Group, 2011).

Commonly recommended combinations include *rbcL* + *matK*, *matK* + ITS, and *rbcL* + *matK* + ITS, with the latter providing the highest identification accuracy. Chloroplast markers ensure phylogenetic stability, while

nuclear markers enhance species-level discrimination (Kress & Erickson, 2007). Recent studies also highlight the potential of integrating *ycf1* and adopting genome skimming or plastome-based approaches to further improve resolution and support more robust evolutionary analyses (Dong et al., 2015; Hollingsworth et al., 2016).

8. Emerging Trends and Future Perspectives

The rapid development of molecular biology and sequencing technologies has significantly advanced DNA barcoding and plant systematics. While traditional barcoding approaches using standard loci such as *rbcL*, *matK*, and ITS have greatly improved species identification and taxonomic resolution in *Euphorbia*, several persistent challenges—including low interspecific genetic divergence, hybridization, polyploidy, and incomplete reference libraries—highlight the need for more advanced genomic strategies (Hollingsworth et al., 2011; Hollingsworth et al., 2016). In response, modern research is increasingly shifting toward high-throughput sequencing (HTS), genome-wide analyses, and integrative taxonomic frameworks that provide deeper and more reliable insights into species boundaries, evolutionary relationships, and biodiversity patterns.

One of the most important emerging approaches is DNA metabarcoding, which combines traditional barcoding with next-generation sequencing to identify multiple species simultaneously from complex environmental samples. Unlike conventional barcoding that targets single organisms, metabarcoding analyzes mixed DNA from sources such as soil, water, pollen, herbivore gut contents, and environmental DNA (eDNA) (Taberlet et al., 2012; Cristescu, 2014). In *Euphorbia*, this technique is particularly useful for large-scale biodiversity assessments, ecological monitoring, and detection of rare or invasive species. However, its accuracy is influenced by limitations such as primer bias, uneven amplification, sequencing errors, and incomplete reference databases.

Another promising advancement is genome skimming, a low-coverage sequencing strategy that retrieves high-copy genomic regions including plastid genomes, mitochondrial DNA, and ribosomal DNA (Straub et al., 2012). Compared with single-locus barcoding, genome skimming provides a much richer dataset while remaining cost-effective. In *Euphorbia*, it enhances species identification, supports phylogenetic reconstruction, and is especially valuable for analyzing degraded DNA from herbarium specimens. This approach has become increasingly important for taxonomic revision and conservation genomics.

Closely related to genome skimming is chloroplast genome barcoding, often referred to as “super-barcoding.” This method utilizes complete plastome sequences rather than a few selected loci, offering significantly higher discriminatory power (Li et al., 2015). Plastome-scale data can resolve complex phylogenetic relationships that remain unresolved using traditional barcode regions. In *Euphorbia*, super-barcoding helps identify mutational hotspots, improves species delimitation, and provides deeper insights into evolutionary history, diversification patterns, and biogeography (Dong et al., 2015).

Next-generation sequencing (NGS) platforms such as Illumina, PacBio, Oxford Nanopore, and Ion Torrent have revolutionized molecular taxonomy by enabling rapid, high-throughput sequencing at reduced cost (Shokralla et al., 2014). These technologies support genome skimming, phylogenomics, transcriptomics, and environmental sequencing applications. In *Euphorbia*, NGS facilitates genome-wide marker discovery, large-scale phylogenetic reconstruction, and comprehensive biodiversity assessments, making it a cornerstone of future taxonomic research.

In addition to technological advances, integrative taxonomy is increasingly recognized as a necessary framework for resolving complex species boundaries. This approach combines molecular, morphological, ecological, cytological, and phylogenetic evidence to produce more robust and biologically meaningful classifications (Dayrat, 2005; Padial et al., 2010). In *Euphorbia*, where morphological convergence and hybridization are common, integrative methods are especially important for accurate species delimitation and evolutionary interpretation.

Overall, the future of DNA barcoding in *Euphorbia* is moving from single-locus identification systems toward genome-scale and integrative approaches. Continued improvements in sequencing technologies, analytical tools, and global reference databases will significantly enhance species identification accuracy, evolutionary research, and conservation planning. These developments will also strengthen the sustainable utilization and medicinal authentication of *Euphorbia* diversity worldwide (Hollingsworth et al., 2016).

9. Conclusion

DNA barcoding has emerged as a robust and widely applied molecular tool for species identification, taxonomic clarification, biodiversity assessment, and conservation biology in the genus *Euphorbia*. Given the genus's exceptional species richness, morphological plasticity, and complex evolutionary history, traditional morphology-

based identification is often insufficient. DNA barcoding provides a standardized, rapid, and reproducible alternative that helps overcome these limitations and improves taxonomic accuracy.

Among commonly used plant barcode markers, chloroplast genes such as *rbcL* and *matK* remain the most widely adopted core barcodes due to their universality and amplification success. While *rbcL* is highly reliable for amplification and higher-level taxonomic placement, *matK* offers greater species-level resolution. Nuclear markers such as ITS and ITS2 provide even higher discriminatory power because of their faster evolutionary rates and greater sequence variability. In addition, *ycf1* has emerged as a promising next-generation barcode due to its high mutation rate and strong phylogenetic signal. Overall, multilocus combinations consistently outperform single-marker approaches in *Euphorbia* species identification.

Despite these advantages, DNA barcoding in *Euphorbia* faces several challenges, including low genetic divergence among closely related species, hybridization, polyploidy, incomplete lineage sorting, and limited reference sequence coverage. Technical constraints such as DNA degradation, PCR amplification bias, and alignment difficulties further complicate analyses and may reduce identification accuracy.

Recent technological advances—including DNA metabarcoding, genome skimming, chloroplast genome sequencing, and next-generation sequencing—are significantly improving resolution and expanding the scope of molecular taxonomy. When integrated with morphological, ecological, and cytological evidence, these approaches form a comprehensive framework for species delimitation and evolutionary studies.

In conclusion, DNA barcoding, particularly when combined with genome-scale data and integrative taxonomic approaches, provides a powerful and evolving framework for understanding the diversity, evolution, and conservation of *Euphorbia*. Continued development of molecular tools and reference databases will further enhance its effectiveness in taxonomy, biodiversity research, and medicinal plant authentication.

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