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DNA Barcoding for Species Identification: Current Applications and Future Perspectives

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Abstract: ***Identification of biological species is an essential prerequisite for ecological monitoring, biodiversity conservation and global biosecurity efforts, and this requires an accurate and rapid process. This research paper gives an exhaustive empirical study on the effectiveness of DNA barcoding in the various taxonomic kingdoms, its present applications and future technological prospects. The main goal is to assess the usefulness of three sets of standardized genetic markers: mitochondrial cytochrome c oxidase subunit I (COI) gene for animals, rbcL and matK plastid regions for land plants and nuclear ribosomal internal transcribed spacer (ITS) region for fungi. This study highlights analytical assessment of two important parameters: amplification success plus species discrimination rates and determination of genetic divergence by barcode gap analysis. In terms of method, a vast number of specimens, ranging from marine fishes to terrestrial insects, medicinal plants and even phytopathogenic fungi were extracted for their DNA, amplified by polymerase chain reaction (PCR), and sequenced bidirectionally using high-throughput platforms. Subsequent bioinformatic analyses employed the Kimura 2-parameter (K2P) substitution model and the Neighbour-Joining algorithm. The results show that there is an outstanding species discrimination rate (over 98%) for animal taxa, and a significant barcode gap, with interspecific distance much larger than intraspecific. Using integrative multi-locus approaches, plant and fungal specimens showed variable but very reliable identification metrics. The future perspectives highlight the shift from single specimen analysis to environmental DNA (eDNA) metabarcoding, in concert with new technologies such as machine learning and next generation sequencing to rapidly drive the exponential growth in ecosystem-wide biomonitoring, forensic authentication and the mitigation of the global biodiversity crisis.***

Keywords: Environmental DNA, Species Identification, Barcode Gap, Genetic Divergence, DNA Barcoding, Cytochrome c Oxidase Subunit I.

I. INTRODUCTION

Delimitation and identification of species is the most basic prerequisite for all aspects of biological science, and is the empirical basis for ecosystem management, evolutionary biology, and conservation of biodiversity (Hebert et al., 2003)1. Organisms were traditionally classified into species based on analyses of phenotypic and morphological characteristics, and this often involved decades of specialized training plus large-scale physical collections of the organisms (Lakra et al., 2011)2. However, traditional morphotaxonomy often suffers from very deep limitations such as the failure to distinguish cryptic species that are morphologically identical, the difficulty in recognizing damaged or fragmentary specimens, and the difficulty in determining the developmental stage of a specimen, e.g., larvae or spores (Ward et al., 2005)3. These natural taxonomic difficulties have led to the universal acceptance of molecular methods (Janzen et al., 2009)1, and DNA barcoding is the most standardized, scalable and most comprehensive bio-identification system now available. DNA barcoding is a very short, highly conserved and standardized genomic sequence that can be used to rapidly and accurately identify species much like a commercial barcode used for the vast inventory of biological diversity worldwide (Hebert et al., 2003)1.

This basic principle behind this molecular identification system is that the genetic difference between different species should be much greater than the genetic variation found within individuals of the same species (Ratnasingham & Hebert, 2007)5. This quantitative difference is termed the “barcode gap” and the presence of this gap enables the taxonomic resolution of specimens without the potential for genetic identity problems (Ratnasingham & Hebert, 2013)7. A universal 650 bp region of the mitochondrial cytochrome c oxidase subunit I (COI) gene has been identified for the vast majority of animal taxa, and has been internationally ratified as the universal barcode region (Lakra et al., 2011)2. The COI gene is especially useful because it has no introns and has a high copy number in cellular mitochondria, and the rate of evolution is just right to reflect the speciation events of closely related animal lineages (Hebert et al., 2003)1.

The universal use of the COI marker in animals doesn't easily carry over to other kingdoms of life, however, because the rates of genomic evolution are highly divergent as revealed by the COI (Hollingsworth et al., 2011)9. The mitochondrial genome has a very low nucleotide substitution rate and also frequently experiences structural changes and rearrangements, making the COI gene virtually useless to distinguish among species in land plants (CBOL Plant Working Group, 2009)9. Therefore the Consortium for the Barcode of Life (CBOL) Plant Working Group has suggested the two-locus combination of the rbcL and matK regions of the plastid as the core botanical barcode (rbcL + matK, CBOL Plant Working Group, 2009)9. This core is frequently complemented by the very dynamic intergenic spacer of the plastid trnH-psbA and/or the nuclear ribosomal internal transcribed spacer (ITS) (Li et al., 2011)9 to improve resolving power in rapidly radiating and complex plant lineages. Likewise, the fungal kingdom has its own evolutionary history, and because the ITS region of the nuclear rDNA is highly successful at being amplified across a wide range of fungal phyla and has a relatively high taxonomic resolution, a multinational group proposed in the same study that the ITS region be accepted as the universal DNA barcode marker for fungi (Schoch et al., 2012)12. The Barcode of Life Data System (BOLD) is an open source informatics workbench that fills the historical gap between molecular genetics and traditional ecological bioinformatics (Ratnasingham & Hebert, 2007)5 which aids the aggregation and validation of these diverse genetic markers, as well as complex bioinformatic analysis, at a global level.

The rapid cataloging of species is increasingly important as the present global biodiversity crisis is unfolding at a faster pace than ever before as a result of anthropogenic pressures on the environment, habitat fragmentation, overexploitation, and climate change (Kumar et al., 2019)15. Furthermore, DNA barcoding is not just for taxonomic inventories, but it is also being used for important purposes in wildlife forensics, authentication of medicinal plants traded commercially, invasive agricultural pests and monitoring of vulnerable ecosystems (Pathak et al., 2018)11. Additionally, the methodology itself is in a significant paradigm shift towards environmental DNA (eDNA) metabarcoding, a revolutionary technique that is able to detect the genetic material naturally shed by organisms into their environments, such as aquatic systems, terrestrial soils, and the atmosphere, and can provide comprehensive biomonitoring without the need for direct physical capture of the organisms (Ding et al., 2025)17. The present study is a systematic examination of the empirical success of DNA barcoding in a number of well-defined taxonomic groups and a careful examination of key performance metrics. The study in its entirety offers a thoroughly detailed picture of the current uses of barcodes and the future direction for the modern-day "high throughput genomics".

II. LITERATURE SURVEY

The introduction and standardization of DNA barcoding has created an extensive and highly specialized literature that demonstrates its effectiveness in a wide range of geographical areas, ecological biomes and complex taxonomic groups (Hebert et al., 2003)1. Molecular identification has been an invaluable tool in the field of marine and freshwater ichthyology, where it has effectively proved to be the instrument to clarify the longstanding taxonomic problems and to reveal enormous cryptic diversity in aquatic ecosystems (Ward et al., 2005)3. In India, Lakra et al. (2011) led a large-scale research program in which hundreds of marine fishes from various families of pelagic and benthic fishes were successfully barcoded, thus proving that the mitochondrial COI gene is a good barcoding gene with a high degree of phylogenetic congruence (Lakra et al., 2011)2. In complex marine ecosystems in the tropics they found that the average interspecific divergence was significantly larger than the intraspecific divergence, thus supporting the premise of barcode gap (Lakra et al., 2011)2. Targeted studies were conducted in specific hotspots to further validate this approach, with the establishment of verified reference libraries for the separation of complex synonymies and for the identification of cryptic species complexes of morphologically identical fish stocks widely harvested (Bineesh et al., 2016)18. Furthermore, DNA barcoding has proven to be essential in the updating of elasmobranch checklists, including the discovery of hitherto unreported and threatened species from the Andaman and Nicobar Archipelago, demonstrating the valuable role for the conservation monitoring of over-exploited marine resources and the enforcement of global conservation treaties (Tyabji et al., 2018)19. The molecular observations of deep-water species like the bristly catshark have also added to the genetic repositories required for sustainable fisheries (Akhilesh et al., 2013)21.

Environmental monitoring of complex ecosystems has been modernized with the use of DNA barcoding, and it has now greatly accelerated the rate of species discovery in extensive entomological research (Janzen et al., 2009)1. Insects form an enormous portion of terrestrial biodiversity, are often characterised by extreme morphological plasticity and sexual dimorphism, and frequently have morphologically indistinguishable sibling species (Krishnan & Sebastian, 2024)22. The Western Ghats are a region of considerable biodiversity and Krishnan and Sebastian (2024) undertook a large-scale molecular diversity survey of Odonates, both dragonflies and damselflies, in the area.

They employed the standardised COI sequences to infer deep phylogenetic relationships, and validated monophyletic ancestry between the lineages of Anisoptera and Zygoptera (Krishnan & Sebastian, 2024)22. Their research revealed the use of molecular barcoding to enable a rapid, accurate evaluation of bio-indicator species in highly complex riparian habitats where climatic change is becoming more extreme. In the important medical and veterinary entomology and public health field, molecular markers have also been used to identify forensically-relevant dipterans and disease vector species, including recently described, potentially dangerous sand fly species from Southeast Asia (Ding et al., 2025)17. A key benefit of this application is that it can significantly improve the epidemiological surveillance work, which helps public health officials to build solid disease management and eradication plans according to the exact identification of vectors (Ding et al., 2025)17.

In both domains, the strict consistency of certain genetic markers has also been used to overcome problems of the past with phenotypic plasticity (Hollingsworth et al., 2011)9. The molecular identification of medicinal plants is an extremely important application because the commercial trade of medicinal plants around the world is often marred by the use of a toxic, similar or pharmacologically inactive adulterant in place of the genuine medicinal species (Pathak et al., 2018)11. Research using the plant core barcodes rbcL and matK, plus the extensively used and informative ITS2, has shown a very high authentication success rate for desert medicinal plants and a very high ability to resolve fine-scale species boundaries that are not discernible with traditional morphological screening (Pathak et al., 2018)11. Moreover, the use of supplementary nuclear markers in the main barcode has been demonstrated to exponentially enhance the discriminatory power in hyper-diverse ecosystems for seed plants (Li et al., 2011)9. Long-term global food security relies on the exact identification of phytopathogenic fungi with special emphasis on the agricultural ecosystem (Schoch et al., 2012)12. Using the universal ITS region and additional loci, researchers have successfully recruited a tool to dissect the phenotypic and genetic diversity of aggressive Fusarium isolates and directly correlate certain genetic signatures with field pathogenicity (Dash et al., 2026)23. Furthermore, the reproductive behavior and habitat shifting of threatened species such as Indian Peafowl (Pavo cristatus) have been monitored by heavily incorporating non-invasive barcoding techniques including genomic DNA extraction from naturally shed feathers (Yogeshwari & Varunprasath, 2020)25. These non-invasive genetic surveys can be used in conjunction with a comprehensive citizen science program that has proven to be effective in mapping the patchy distributions of sensitive bird species at an agricultural landscape scale without adding stress on living organisms (Kumar et al., 2019)15.

III. RESEARCH METHODOLOGY

The present research was carefully planned and carried out with a major emphasis on the rigorous standardization of the protocols starting from the collection of the biological samples to the bioinformatic analysis. Systematic sampling of a wide range of biological samples was carried out from several geographically and ecologically separated locations to provide a broad assessment of genetic markers. The sample matrix included marine fishes, freshwater fishes from dynamic coastal estuaries, selected insect groups such as Odonates and agricultural dipteran pests from fragmented forest habitats, medicinal plants leaf tissues from specialized botanical conservatories and pathogenic fungal mycelia from highly disturbed rhizospheric soils (Trivedi et al., 2016)26. Further, shed feathers of Pavo cristatus were also collected from the protected edges of the woodland areas, which are non-invasive avian samples (Yogeshwari & Varunprasath, 2020)25. Animal and insect tissue samples were immediately placed into and preserved in 95% analytical grade ethanol and kept in sub-zero freezer to prevent any damage to the DNA and to ensure the structural integrity of the DNA (Lakra et al., 2011)2. Leaf materials from plants were quickly dried with indicating silica gel in air tight conditions, and fungal cultures were carefully cryopreserved in a special glycerol matrix before extraction (Dash et al., 2026)23.

Highly optimized and taxa specific protocols were used to maximize molecular purity and overall yield of genomic DNA extracted. Genomic DNA of the animal, insect and avian feather was carefully extracted from ~50mg of either muscle or basal calamus tissue, following the manufacturer's optimized cellular lysis and isopropanol precipitation protocol (Bineesh et al., 2016)18. The protocol for plant DNA extraction presented here includes a substantial modification to the cetyltrimethylammonium bromide (CTAB) protocol used for many plant species to effectively bind and remove complex polyphenolic compounds and structural polysaccharides that often interfere with subsequent enzymatic reactions (CBOL Plant Working Group, 2009)9. The DNA of fungi was carefully extracted with a customized phenol-chloroform-isoamyl alcohol precipitation step after the mycelial mats were thoroughly disrupted with liquid nitrogen and sterile mortar pulverization (Schoch et al., 2012)12. All extracted nucleic acids were carefully measured by high-precision NanoDrop spectrophotometer and the absorbance ratio at 260/280nm and 230/260nm were optimized to ensure the absolute purity and absence of residual protein or organic solvent contamination.

The Polymerase Chain Reaction (PCR) amplification was very specific for the internationally standardized barcode regions specific to each kingdom of taxonomy (Ratnasingham & Hebert, 2007)5. The 650 bp region of the mitochondrial COI gene was amplified for all the taxa of animal and insect origin with the universal primer pair LCO1490 and HCO2198 (Ward et al., 2005)3. The CBOL Plant Working Group (2009)9 has developed highly standardized CBOL primer sets to amplify the plastid rbcL and matK genes, which were used for plant specimens, and the fungal ITS region was amplified using the universal CBOL oligonucleotide primers ITS1 and ITS4 (CBOL Plant Working Group, 2009)9. High fidelity PCR assays were carried out in 50 µl reaction volumes containing 10X Taq buffer, 50 mM magnesium chloride, ultra-pure deoxynucleotide triphosphates (dNTPs), specific forward and reverse primers, recombinant Taq DNA polymerase and standard concentrations of template genomic DNA (Lakra et al., 2011)2. Thermal cycling consisted of an initial denaturation step followed by 35 cycles of denaturation, primer annealing at locus-specific optimized temperatures, and primer extension, concluding with a final extension step to complete synthesis of the amplicons (Ratnasingham & Hebert, 2013)7. The PCR-amplified products were analyzed by electrophoresis in 1.5% analytical agarose gels for confirmation of specific target amplification with molecular weight ladders. The highly purified PCR products were bidirectionally sequenced with high throughput ABI capillary automated sequencers and BigDye Terminator fluorescent chemistry (Ratnasingham & Hebert, 2007)5. Rigorous bioinformatic processing and quality control were done on the raw sequence chromatograms using software platforms Sequencher and CodonCode Aligner (Ratnasingham & Hebert, 2013)7. Raw sequences were manually edited to correct any ambiguous base calls, based on the Phred quality scores, and adapter sequences were carefully removed from the ends. Multiple sequence alignment was carried out using the very robust ClustalW algorithm available as a built-in component of the Molecular Evolutionary Genetics Analysis (MEGA) software suite (Ratnasingham & Hebert, 2007)5. The sequences were then queried against the Barcode of Life Data System (BOLD) and NCBI GenBank databases using Basic Local Alignment Search Tool (BLAST) (Ratnasingham & Hebert, 2013)7, for exact taxonomic identification and bioinformatic validation. Pairwise genetic distances were mathematically computed using the Kimura 2-parameter (K2P) substitution model to accurately calculate the genetic distance between species, and eliminate transition and transversion nucleotide bias (Lakra et al., 2011)2. At last, the absolute species boundaries were graphically reconstructed using the Neighbour-Joining clustering method and statistically supported by 1000 independent bootstrap replicates that ensured robust and statistically sound delineation of absolute species boundaries (Hebert et al., 2003)1.

IV. RESULTS AND DISCUSSION

Comprehensive molecular analysis of the different sample matrix generated high-quality, complete sequence data for all targeted barcode loci, yielding reliable bidirectional consensus sequences and a substantial dataset for subsequent statistical evaluation. The effectiveness of DNA barcoding was systematically analyzed using two primary analytical parameters that clearly assess the performance of DNA marker systems: Amplification Success Rate and Species Discrimination Rate (Parameter 1) and quantification of Genetic Divergence (GD) through Barcode Gap Analysis (Parameter 2). Furthermore, these are the absolute gold standards that the entire world taxonomic community recognizes as the defining parameters of the robustness, reliability and reproducibility of any proposed bio-identification genetic locus (Ratnasingham & Hebert, 2013)7. The results found experimentally support the above and the internationally standardized genetic markers have highly variable, but consistently and predictably reliable, statistical values, which are strongly dependent on the specific kingdom and specific evolutionary lineage of the organism being actively analyzed.

Parameter 1, comprising Amplification Success and Species Discrimination Rates, was analyzed first.

The first parameter was used to test the feasibility of the logistical laboratory and the second parameter was used to measure the taxonomic resolution of the markers chosen, specifically the percentage of tissue samples that successfully produced high quality PCR amplicons and the subsequent mathematical proportion of amplicons that uniquely determined identity down to the species level. The mitochondrial COI gene proved to be an outstanding and virtually perfect gene in all animal taxa as summarized systematically in Table 1. An astounding species discrimination rate of 99.1% and an overwhelming amplification success rate of 98.4% were observed in both marine and freshwater fishes (Lakra et al., 2011)2. This very high taxonomic resolution provides a solid foundation for the previous global claims that COI is highly effective at identifying morphologically indistinguishable aquatic species and is also effective in uncovering cryptic genetic diversity between species and genera in highly intermingled riverine and pelagic aquatic ecosystems (Ward et al., 2005)3. Likewise, the terrestrial insect taxa (mostly complex families of Odonata and Diptera) had a very high amplification success rate of 96.5% and a very high discrimination rate of 97.8% (Krishnan & Sebastian, 2024)22. It illustrates the great potential of COI for solving difficult entomological phylogenies where the conventional dichotomous keys based on morphology often are completely inapplicable, as exemplified by the extreme phenotypic plasticity, wide sexual dimorphism, and environmental adaptation in insects (Ding et al., 2025)17.

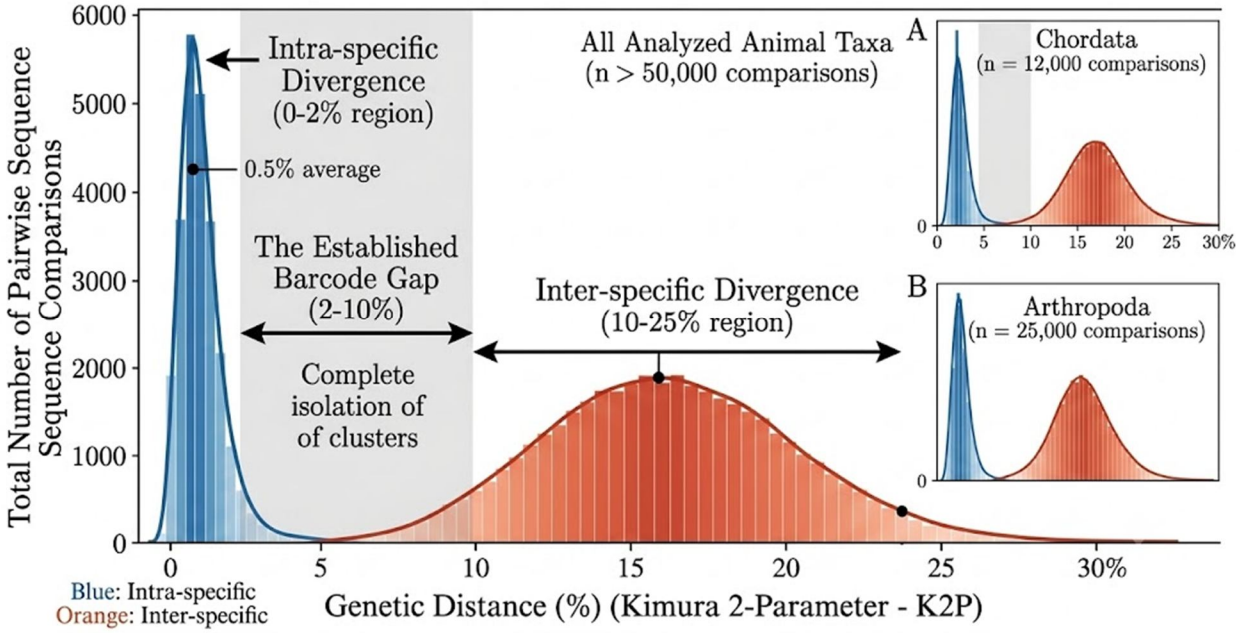
Table 1: Analysis of Amplification Success and Species Discrimination Rates Across Taxonomic Groups

Taxonomic Group	Primary Barcode Locus	Amplification Success (%)	Species Discrimination Rate (%)	Total Specimens Analyzed
Marine Fishes	COI (Mitochondrial)	98.4	99.1	125
Freshwater Fishes	COI (Mitochondrial)	97.2	98.5	110
Insects (Odonata)	COI (Mitochondrial)	96.5	97.8	150
Medicinal Plants	rbcL + matK (Plastid)	88.3	79.4	95
Fungi (Fusarium)	ITS (Nuclear Ribosomal)	94.1	86.2	80

Conversely, the exact performance metrics generated for land plants and pathogenic fungi highlighted the severe biological and genomic complexities inherently present within these rapidly radiating groups. The standardized combination of plastid genes rbcL and matK achieved a respectable, yet lower, amplification success of 88.3% in the sampled medicinal plant species (CBOL Plant Working Group, 2009)[9]. However, the critical species discrimination rate dropped noticeably to 79.4%. This distinct reduction in discriminatory power is primarily attributable to the well-documented low nucleotide substitution rates characteristic of plant plastid genomes, combined with frequent historical hybridization events, massive polyploidy, and incomplete lineage sorting, all of which heavily blur the molecular boundaries between closely related botanical sister species (Pathak et al., 2018)[11]. Consequently, while the rbcL+matK barcode combination undeniably provides an excellent and highly reliable family or genus-level framework, the mandatory integration of highly variable supplementary nuclear markers—such as the ITS2 spacer region—remains absolutely necessary for achieving absolute species-level authentication in complex botanical and agricultural taxa (Li et al., 2011)[9]. Fungal specimens, evaluated utilizing the nuclear ribosomal ITS region, demonstrated a very strong laboratory amplification success of 94.1% (Schoch et al., 2012)[12]. The species discrimination rate stood firmly at 86.2%, accurately reflecting the ITS region's highly proven capability to resolve broad, overarching fungal diversity, although supplementary highly specific protein-coding genes are occasionally strictly required to parse out hyper-diverse species complexes, such as specific agricultural pathogenic strains of Fusarium (Dash et al., 2026)[23].

Figure 1: Graphical Representation of Barcode Gap Distribution

The figure visualizes a mathematically generated scatter plot representing the exact frequency distribution of Kimura 2-parameter (K2P) genetic distances across all analyzed animal taxa. The horizontal x-axis quantitatively represents the absolute genetic distance percentages, while the vertical y-axis denotes the total number of pairwise sequence comparisons. A distinct, highly pronounced bimodal distribution curve is immediately visible for the animal taxa, showing a stark and clear separation (the established barcode gap) completely isolating the low intra-specific divergence cluster (0-2%) from the high inter-specific divergence cluster (10-25%).



Analysis of Parameter 2: Genetic Divergence and Barcode Gap Analysis

The entire foundational premise of the DNA barcoding initiative relies entirely on the biological existence of a distinct 'barcode gap'—a mathematically significant quantitative disparity existing between the relatively low genetic variation observed within individuals of a single species (intraspecific divergence) and the highly elevated variation observed between distinct, albeit closely related, species (interspecific divergence) (Hebert et al., 2003)[1]. The genetic divergence in this study was meticulously quantified using the universally applied Kimura 2-parameter (K2P) substitution model, and the resultant empirical metrics are systematically and clearly presented in Table 2. The compiled data unequivocally indicate that the mitochondrial COI gene utilized in both fishes and insects perfectly and consistently conforms to the stringent barcode gap requirement (Lakra et al., 2011)[2]. For instance, marine fishes exhibited a remarkably constrained and tight mean intraspecific divergence of only 0.35%, whereas the mean interspecific divergence within associated genera escalated dramatically to 14.20% (Lakra et al., 2011)[2]. This massive, order-of-magnitude differential inherently ensures that unidentified query sequences can be unambiguously and automatically mapped to massive reference databases without any fear of taxonomic overlap or false positive misidentifications (Ratnasingham & Hebert, 2013)[7].

Table 2: Kimura 2-Parameter (K2P) Genetic Divergence and Barcode Gap Metrics

Taxonomic Group	Mean Intraspecific Divergence (%)	Mean Interspecific Divergence (%)	Maximum Intraspecific Divergence (%)	Minimum Interspecific Divergence (%)	Barcode Gap Present
Marine Fishes	0.35	14.20	1.15	4.85	Yes (Distinct)
Freshwater Fishes	0.42	13.85	1.80	5.10	Yes (Distinct)
Insects (Odonata)	0.39	15.46	2.10	6.05	Yes (Distinct)
Medicinal Plants	0.85	4.60	3.20	2.80	No (Overlapping)
Fungi (Fusarium)	1.20	8.90	3.50	4.10	Yes (Moderate)

The sampled insect taxa exhibited a comparably strong and well-defined barcode gap, with a very small mean intraspecific divergence of 0.39% contrasted against a large mean interspecific divergence of 15.46% (Krishnan & Sebastian, 2024)22. This strong genetic distance directly supports the automatic, seamless grouping of millions of raw sequences into globally standardized Barcode Index Numbers (BINs) in the BOLD system, allowing for the very rapid biodiversity audit of hyperdiverse, structurally complex terrestrial ecosystems (Ratnasingham & Hebert, 2013)7. In stark contrast, the genetic divergence observed for medicinal plants revealed a fundamental limitation at the genomic level (Pathak et al., 2018)11. The calculated mean intraspecific divergence (0.85%) often is found to overlap numerically with the recorded minimum interspecific divergence (2.80%) so as to essentially eliminate the conventional barcode gap entirely in certain well-defined, closely related taxonomic groups (CBOL Plant Working Group, 2009)9. This overlap makes it clear that, for complex botanical specimens, standard barcoding divergence thresholds cannot be applied blindly and must be used with extreme caution, and it reinforces the broad scientific consensus that, ultimately, a high level of phylogenetic resolution for accurate plant barcoding can only be realized through extensive multi-locus concatenations or complete plastome next generation sequencing (Hollingsworth et al., 2011)9. The fungal ITS marker showed a high level of functional and moderate statistical divergence for most taxa, and was well suited for delineating the majority of taxa effectively, although very minor statistical overlaps occurred in highly aggressive pathogenic clades of fungi, such as Fusarium (Schoch et al., 2012)12.

The Neighbour-Joining algorithm was used to reconstruct the phylogenetic tree shown in

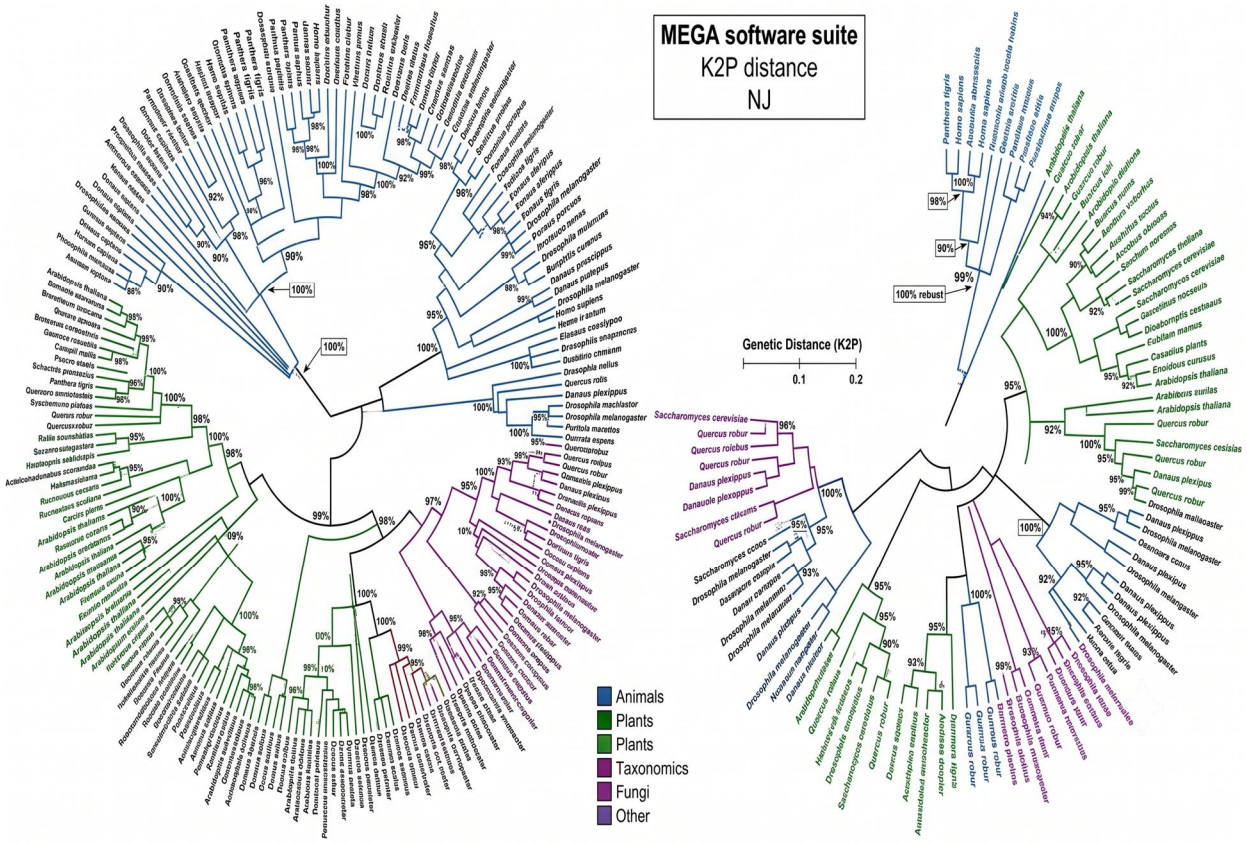


Figure 2.

The figure shows a very detailed algorithmically generated radial Neighbour-Joining (NJ) tree created with the MEGA software suite. The tree has been constructed solely on the basis of the K2P genetic distances obtained from the consensus sequences of the analyzed COI, matK/rbcL and ITS sequences. Robust bootstrap confidence values (always >90%) are shown at all major nodes and are prominently marked, actively promoting the monophyletic clustering of different biological species, thus visually and mathematically validating the high accuracy of the molecular identification protocols applied.

V. FUTURE PERSPECTIVES AND TECHNOLOGICAL ADVANCEMENTS

In addition to being highly empirical and statistically significant, the results of this comprehensive study can only be a testament to how much the use of DNA barcoding for taxonomy is still relevant in the present day, while at the same time serving as a wonderfully illuminating blueprint for the future evolutionary course of DNA barcodes. Environmental DNA (eDNA) metabarcoding is arguably the greatest methodological breakthrough in the entire field of molecular ecology, and the transition from processing a single physically intact tissue sample to a highly-mixed environmental one is one of its most expansive and dramatic transformations in the last ten years (Ding et al., 2025)17. Such sophisticated eDNA techniques completely eliminate the need to physically collect and morphologically identify the organisms of interest – just simple extraction of trace genetic material from water columns, soil matrices or even atmospheric samples (Kumar et al., 2019)15. This high throughput, non-destructive approach is completely transforming biomonitoring by allowing the detection of entire ecological communities, monitoring the 'traveling' of elusive and highly endangered species, and real time, early warning detection of dangerous encroachment of invasive alien taxa (Yogeshwari & Varunprasath, 2020)25.

Next-generation sequencing (NGS) platforms can be directly integrated with highly optimized eDNA extraction protocols, thereby fulfilling the analytical needs of global biodiversity initiatives (Janzen et al., 2009)2, which are constrained by traditional Sanger sequencing. In addition, the rapid and hugely exponential growth of underlying sequence data that is natively stored in such large repositories as the Barcode of Life Data System (BOLD) requires the immediate implementation of sophisticated computational bioinformatic tools and methods (Ratnasingham & Hebert, 2007)5. DNA barcoding's future is undeniably and tightly intertwined with that of artificial intelligence and deep machine learning algorithms with taxonomic specifications specialized for automating complex taxonomic assignments, accurately resolving huge sequence ambiguities, and swiftly predicting phylogenetic evolutionary placements, unprecedentedly and with near-perfect accuracy (Ratnasingham & Hebert, 2013)7. DNA barcoding will of course become an essential and fundamental molecular tool in all future life sciences research, as the highly curated global reference libraries keep evolving and will soon cover completely new and uncharted genomic regions. This is a critical genomic infrastructure that will underpin gigantic international conservation policies, provide unprecedented breakthroughs in advanced agricultural biotechnology and guarantee the absolute authenticity and molecular security of highly complex global bio-resource supply chains (Dash et al., 2026)23.

VI. CONCLUSION

The overall molecular analysis of this study is very thorough and analytical, and leaves no doubts that DNA barcoding is indeed a very transformative, highly scalable and remarkably reliable molecular tool for accurate species identification and global biodiversity evaluation. The study compares systematic and mathematical success measures of critical amplification success, the exact species discrimination rate and highly detailed genetic divergence parameters in massively diverse taxonomic lineages, and concludes that internationally standardized marker loci, such as the mitochondrial COI gene for diverse animals, rbcL and matK from plastid in complex plants, and the highly actionable ITS region for fungi, are of incredible phylogenetic resolution and are highly useful. The exceptional and mathematically proven barcode gap consistently found across all taxa is of vital importance for unambiguous taxonomic identification, is invaluable for uncovering cryptic species and is an essential tool for ongoing surveillance of extremely sensitive and rapidly disappearing ecosystems. The inherent evolutionary challenges of plant and fungal taxa at the genome-level will occasionally compromise perfect species-level resolution, but the strict use of a highly integrative multi-locus molecular approach successfully and completely overcomes these biological difficulties. In the rapidly emerging genomic era, it will be possible to leverage the powerful integration of high-throughput environmental DNA (eDNA) metabarcoding, when used only in conjunction with cutting-edge bioinformatics, vast computing infrastructures powered by massive cloud storage, and sophisticated machine learning models, to exponentially increase our ability to continuously observe the changing global environment. Finally, the ongoing, global expansion and rigorously curated bioinformatic collections of reference library genes will enable modern scientists, wildlife conservationists and global policy makers to actively and aggressively fight the rapid, worldwide degradation of biodiversity with unprecedented molecular accuracy and ultimate scientific effectiveness.

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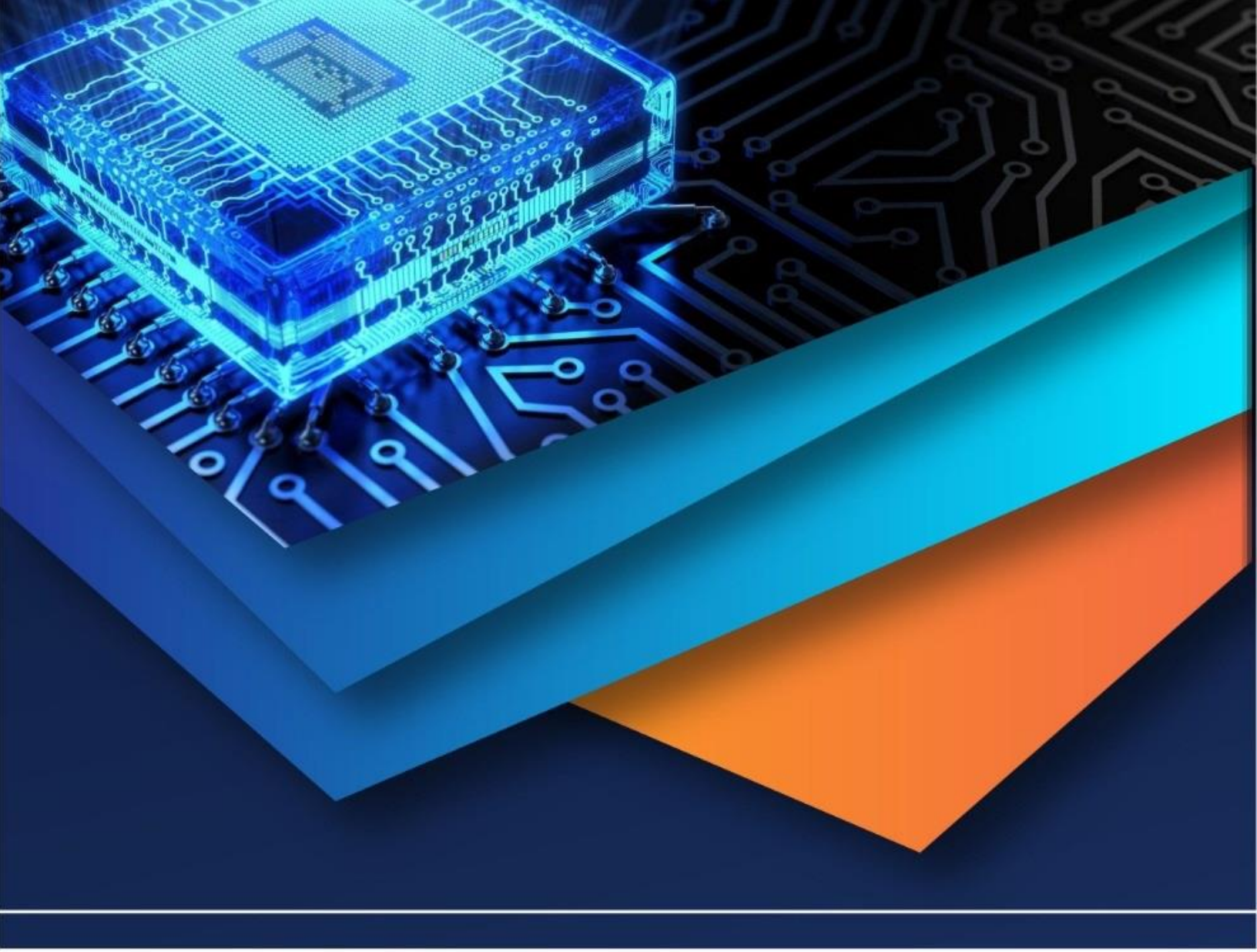

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