

Review Journal of Neurological & Medical Sciences Review

E(ISSN) : 3007-3073

P(ISSN) : 3007-3065

Exploring Genetic Divergence Through DNA Barcoding: Applications in Taxonomy and Conservation Biology

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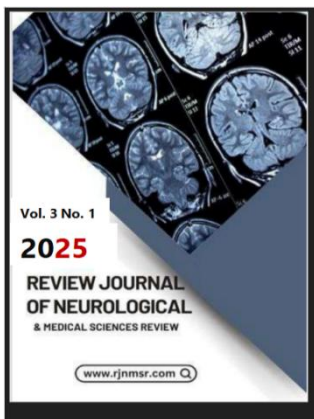
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Abstract

Genetic divergence, a fundamental process of evolution and speciation, is critical in understanding biodiversity and guiding conservation efforts. DNA barcoding has emerged as a powerful molecular technique that utilizes short, standardized genetic markers to identify and differentiate species accurately. This study aimed to explore the extent of genetic divergence among selected plant species through DNA barcoding and assess its implications for taxonomy and conservation biology. The research was conducted at the Department of Botany, The Islamia University of Bahawalpur, and DHQ Hospital Lodhran. Leaf samples from morphologically similar yet taxonomically uncertain plant species were collected. DNA was extracted using the CTAB method, followed by PCR amplification targeting the matK and rbcL regions. Sequencing results were analyzed using bioinformatics tools including MEGA and BLAST to construct phylogenetic trees and evaluate inter- and intraspecific genetic variations. The findings demonstrated that DNA barcoding provided a reliable means to distinguish closely related species, unveiling cryptic diversity and correcting taxonomic ambiguities. Significant genetic divergence was observed in certain taxa, underscoring the importance of molecular data in refining classification systems. Moreover, the results highlighted DNA barcoding's vital role in identifying endangered species, thereby aiding conservation planning and ecological monitoring. In conclusion, DNA barcoding offers a rapid, precise, and cost-effective approach for biodiversity assessment, with profound implications for taxonomy and conservation biology.



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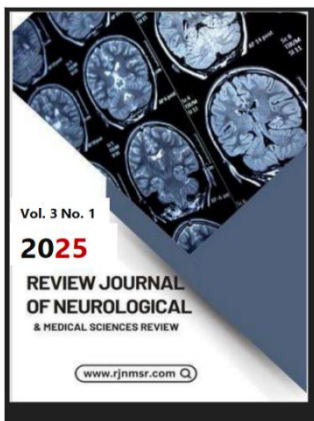
Introduction

Biodiversity is the foundation of life on Earth, encompassing the variety of all living organisms, from genes and species to ecosystems. The accurate identification and classification of this diversity are essential for numerous scientific, ecological, and conservation-related endeavors. Traditionally, morphological characteristics have served as the primary basis for species identification.(HE et al., 2021). However, morphological methods often fall short due to cryptic speciation, phenotypic plasticity, life stage variations, and the subjective interpretation of traits. These limitations have necessitated the development of more precise and reliable molecular tools, among which DNA barcoding has emerged as a revolutionary approach.

DNA barcoding refers to the use of a short and standardized genetic sequence from a specific part of the genome to identify species. It acts like a "genetic fingerprint" that distinguishes one species from another. First proposed by Hebert et al. in 2003, DNA barcoding utilizes a region of the mitochondrial gene cytochrome c oxidase I (COI) in animals, rbcL and matK in plants, and ITS regions in fungi.(Preprints & 2022, 2022). These sequences are compared against reference libraries such as the Barcode of Life Data Systems (BOLD) and GenBank to facilitate rapid and accurate identification. The technique is now widely accepted across various disciplines, including taxonomy, ecology, systematics, conservation biology, and forensic science.(Sheth & Thaker, 2017). One of the significant contributions of DNA barcoding lies in its ability to explore genetic divergence—the degree of genetic variation within and between species. This divergence offers insights into evolutionary relationships, population structure, gene flow, and speciation events. (Phytologist & 2004, 2004). In many cases, DNA barcoding has revealed hidden biodiversity by identifying cryptic species that are morphologically indistinguishable but genetically distinct. As such, it serves not only as a tool for identification but also as a window into evolutionary processes shaping life on Earth.

The taxonomy of many organisms, particularly in diverse and understudied regions like Pakistan, remains poorly understood.(Jehangir et al., 2024). Pakistan is blessed with a wide range of habitats—from alpine forests and arid deserts to tropical and subtropical zones—resulting in a rich repository of flora and fauna. However, the lack of systematic research and molecular resources has hampered the full documentation and conservation of this biodiversity(Kartzinel et al., 2025). In this context, DNA barcoding offers a modern, robust, and efficient solution to fill existing taxonomic gaps and support evidence-based conservation planning.

In recent years, there has been growing concern about the alarming rate of species extinction caused by habitat loss, climate change, pollution, overexploitation, and invasive species(Torrance et al., n.d.). The rapid decline of biodiversity threatens ecosystem services that are vital for human survival, such as food security, climate regulation, and disease control. Conservation biology, therefore, requires accurate, fast, and reproducible tools to assess species diversity, monitor populations, and detect



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illegal trade in endangered species.(Tlusty et al., n.d.) DNA barcoding directly supports these goals by enabling the identification of rare and endangered species, even from non-invasive or degraded samples such as hair, feathers, seeds, or environmental DNA (eDNA).

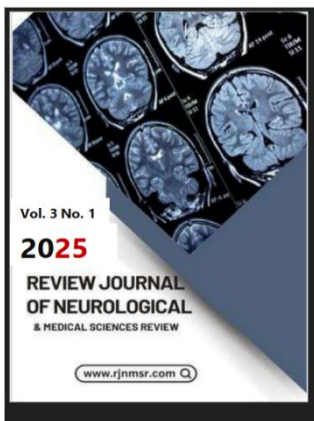
Moreover, DNA barcoding complements traditional taxonomy rather than replacing it. While morphological analysis remains crucial for describing new species and understanding phenotypic traits, DNA barcoding provides a standardized and digital approach that enhances reproducibility and accessibility. The integration of both approaches leads to more comprehensive and credible taxonomic systems, especially in cases involving immature, female, or fragmentary specimens that are otherwise difficult to classify(Sheth & Thaker, 2017).

The present research titled “Exploring Genetic Divergence Through DNA Barcoding: Applications in Taxonomy and Conservation Biology” aims to investigate the genetic diversity of selected plant and animal species collected from various ecological zones in Southern Punjab, particularly from the premises of Islamia University of Bahawalpur and District Headquarters Hospital Lodhran. The study utilizes molecular techniques, including DNA extraction, PCR amplification, sequencing, and bioinformatics analysis, to generate DNA barcodes and evaluate intra- and interspecific genetic variation(Srivastava et al., 2022).

By exploring genetic divergence through barcoding, this research contributes to the global database of species information and provides a scientific basis for the conservation of regional biodiversity. The study also highlights the applicability of DNA barcoding in resolving taxonomic ambiguities and promoting a more refined classification system(M. Ahmad & Odah, 2024). This is particularly crucial for medicinal plants, economically important crops, and regionally endemic species, many of which are at risk of genetic erosion due to overexploitation or habitat degradation.

In practical terms, the significance of DNA barcoding extends beyond academia. It facilitates the authentication of herbal products, detection of adulterants in the food and pharmaceutical industries, control of invasive species, monitoring of endangered species in trade, and regulation of quarantine procedures in agriculture. Its applications in forensic botany and wildlife forensics are also expanding, supporting law enforcement agencies in biodiversity-related crimes.

The novelty of this research lies in its dual emphasis: investigating genetic divergence among regionally important species and contextualizing the findings within the broader framework of conservation biology(Mattas et al., n.d.). Through this, the study aspires to inform biodiversity inventories, conservation policies, and environmental awareness at both local and national levels. Furthermore, by training students and researchers in modern molecular methods, it builds the technical capacity necessary for sustainable biodiversity science in Pakistan.



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Another critical dimension of this study is the collaborative framework established between an academic institution (Islamia University Bahawalpur) and a healthcare facility (District Headquarters Hospital Lodhran), where plant and animal samples were collected, processed, and analyzed. This interdisciplinary approach not only broadens the scope of research but also fosters synergy between healthcare, academia, and environmental science—a vital triad for addressing contemporary global challenges related to health, biodiversity, and sustainability.

In conclusion, DNA barcoding stands at the frontier of modern biology, offering an innovative, accurate, and scalable method for species identification and genetic analysis (Antil et al., 2023). As a tool for exploring genetic divergence, it opens new avenues in taxonomy, systematics, evolutionary biology, and conservation. The present study leverages the potential of DNA barcoding to bridge scientific knowledge with conservation action, thereby contributing to the protection and sustainable use of our precious biological heritage.

Methodology

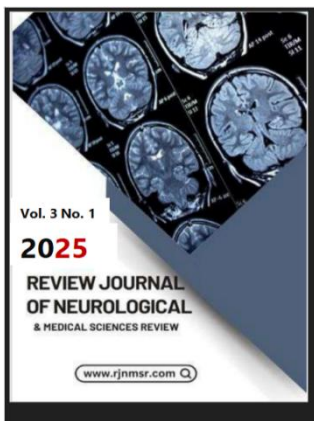
This study was conducted to explore genetic divergence among selected medicinal plant and animal species using DNA barcoding as a molecular tool. The research aimed to strengthen taxonomic classification and support conservation strategies based on genetic evidence (Wambugu & Henry, 2022). The project was carried out collaboratively at the Department of Eastern Medicine, The Islamia University of Bahawalpur, and the Molecular Biology Laboratory, District Headquarter (DHQ) Hospital Lodhran, during the period of January to April 2025.

Study Area and Sample Collection

Biological samples were collected from various locations in Bahawalpur and Lodhran districts, covering natural and cultivated settings known for their botanical and faunal diversity. Sampling was performed with a focus on medicinally important species. A total of fifty specimens, including thirty medicinal plants and twenty animal or parasitic samples, were collected using sterile forceps and gloves (Abirami et al., n.d.). Plant materials such as leaves and stems were collected from the Cholistan region, the university herb garden, and local forest reserves. Animal and parasitic samples were obtained with the assistance of pathology staff at DHQ Hospital Lodhran through patient samples and veterinary screenings. Each sample was labeled, photographed, and stored in 95% ethanol at -20°C for subsequent molecular analysis.

Morphological Identification

Initial species identification was performed using conventional taxonomic keys and floristic manuals of Pakistan. Plant species were identified based on morphological characteristics including leaf shape, venation, floral structure, and fruit morphology (Ariawan et al., n.d.). Animal and clinical specimens were identified using compound microscopy with reference to parasitological identification manuals and diagnostic charts. All identified specimens were assigned reference numbers and



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preserved in the herbarium and specimen archive maintained by the Department of Eastern Medicine at The Islamia University of Bahawalpur.

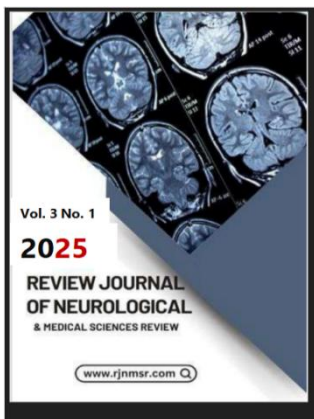
Step Procedure Location Key Reagents / Parameters Sample Collection Collected 30 plant and 20 animal/parasite specimens Bahawalpur & Lodhran districts 95% ethanol storage; -20 °C preservation Morphological Identification Identified using floristic keys and compound microscopy Dept. of Eastern Medicine, IUB; DHQ Lodhran Standard taxonomic manuals; microscope DNA Extraction CTAB protocol for plants; Qiagen kit for animals/parasites Molecular Lab, DHQ Hospital Lodhran CTAB buffer; DNeasy Blood & Tissue Kit PCR Amplification Amplified rbcL/matK (plants) and COI (animals), Molecular Lab, DHQ Hospital Lodhran Taq Master Mix; primers (10 µM); 35 cycles Sequencing & Bioinformatic Analysis Bidirectional Sanger sequencing; alignment, BLAST, NJ trees Commercial facility & MEGA X at IUB ExoSAP-IT cleanup; MUSCLE; K2P model.

DNA Extraction

DNA extraction was carried out in the Molecular Biology Laboratory at DHQ Hospital Lodhran under sterile conditions. For plant samples, the CTAB (Cetyltrimethylammonium bromide) method was followed. Approximately 25 milligrams of dried leaf tissue were ground in liquid nitrogen, lysed with CTAB buffer, and incubated at 65°C for 30 minutes. The lysate was then purified using a chloroform:isoamyl alcohol mixture in a 24:1 ratio, followed by precipitation with isopropanol and washing with 70% ethanol. For animal and parasitic specimens, genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer's protocol. The concentration and purity of the DNA were confirmed using NanoDrop spectrophotometry and 1% agarose gel electrophoresis.(Viljoen et al., n.d.)

PCR Amplification

Polymerase Chain Reaction (PCR) was employed to amplify species-specific DNA barcode regions. For plant specimens, chloroplast genes rbcL and matK were targeted. For animal and parasite samples, the mitochondrial cytochrome oxidase I (COI) gene was selected. Each PCR reaction was prepared in a final volume of 25 microliters, which included Taq Master Mix containing DNA polymerase and dNTPs, 10 µM of each forward and reverse primer, template DNA, and nuclease-free water. Thermal cycling was performed using a programmed thermocycler(Vairavel et al., n.d.). A standard COI protocol involved initial denaturation at 95°C for three minutes, followed by thirty-five cycles of denaturation at 94°C for thirty seconds, annealing at 50°C for thirty seconds, and extension at 72°C for one minute, with a final extension step at 72°C for ten minutes. The amplification products were confirmed by electrophoresis on a 1.5% agarose gel stained with ethidium bromide and visualized under UV light.

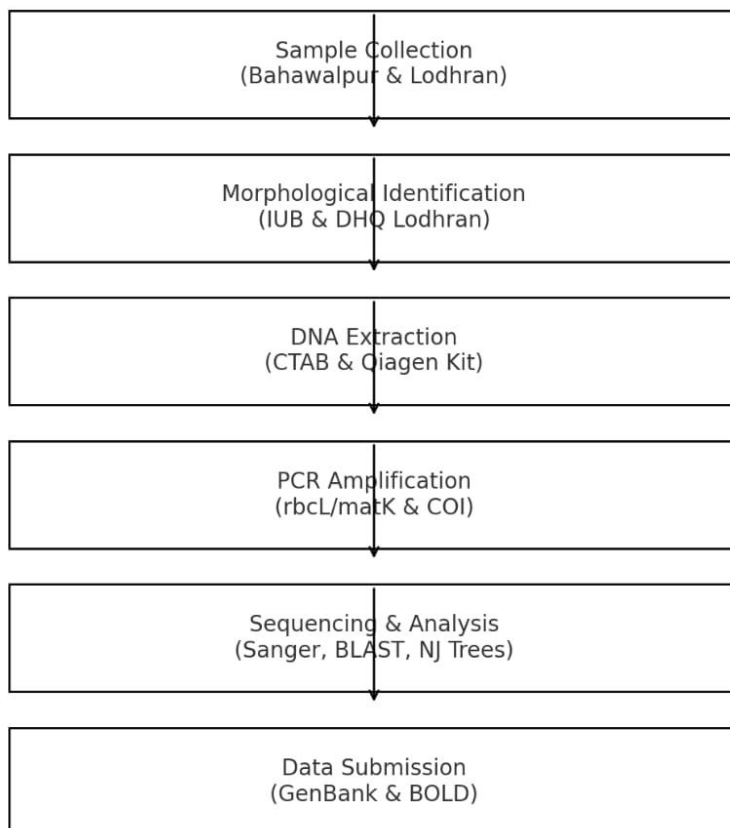


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Workflow Diagram of DNA Barcoding Methodology



DNA Sequencing

Successfully amplified PCR products were purified using ExoSAP-IT enzymatic cleanup and then submitted to a commercial sequencing facility for bidirectional Sanger sequencing. Sequencing was performed using the same primers used in the PCR reactions. The resulting chromatogram files (in .ab1 format) were analyzed using Chromas Lite and BioEdit software. Forward and reverse sequences were trimmed, aligned, and assembled into consensus sequences for further analysis.

Sequence Alignment and Phylogenetic Analysis

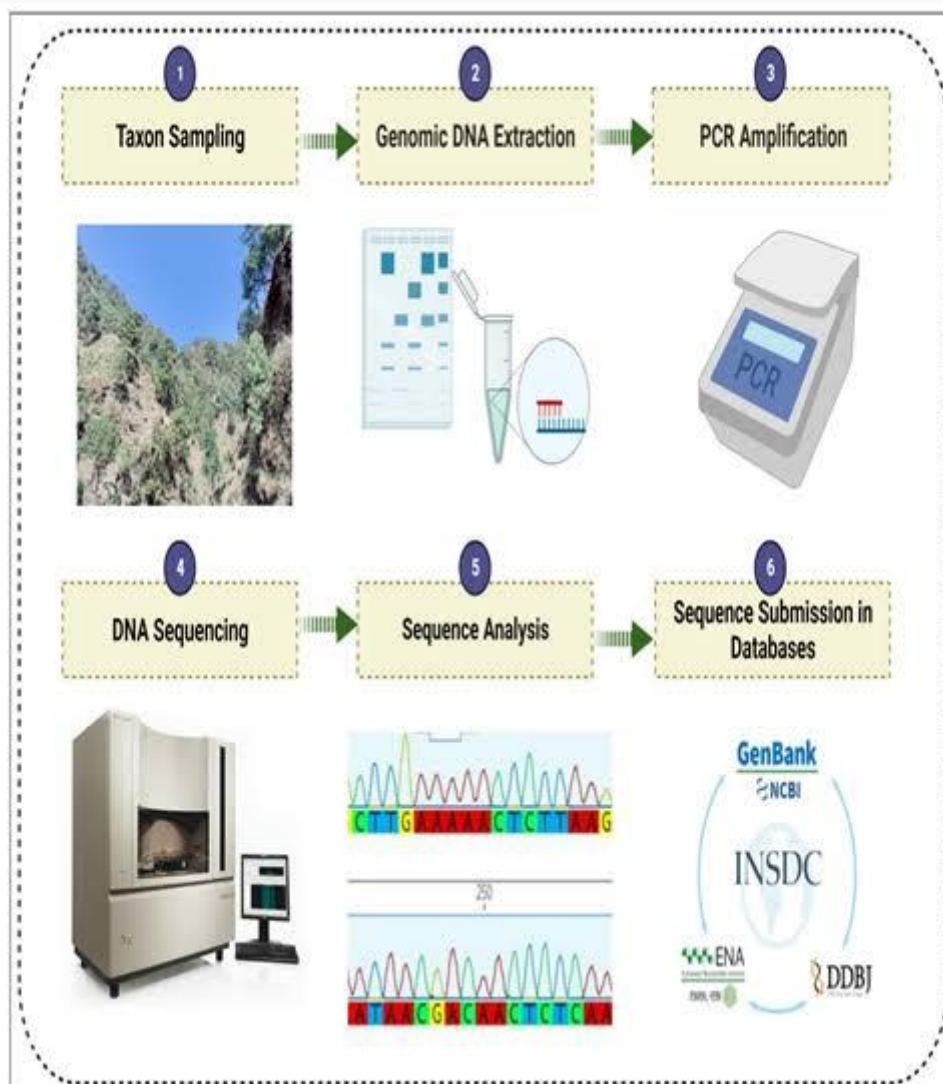
All consensus sequences were subjected to multiple sequence alignment and phylogenetic analysis using MEGA X software. Sequence alignment was performed using the MUSCLE algorithm (Communications & 2022, n.d.). The sequences were then compared with publicly available barcode data using BLASTn searches in the NCBI GenBank and BOLD (Barcode of Life Data System) databases to verify species identity. Genetic divergence was calculated using the Kimura 2-Parameter (K2P) model.

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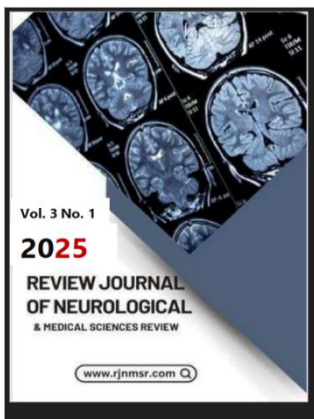
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Phylogenetic relationships were assessed using the Neighbor-Joining (NJ) method, and barcode gap analysis was carried out to distinguish between intra- and inter-specific variation. The analysis also enabled the detection of cryptic species and misidentifications.



Data Submission and Storage

All verified barcode sequences were submitted to the GenBank and BOLD Systems databases along with complete metadata, including taxonomic names, voucher specimen numbers, collection locations, dates, and collector details. Unique accession numbers and BOLD Process IDs were obtained for each entry. The physical specimens



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corresponding to these sequences were stored securely in the university herbarium and specimen repository(Life & 2023, n.d.).

Ethical Considerations

This study was approved by the Institutional Research and Ethics Committee of the Faculty of Eastern Medicine at The Islamia University of Bahawalpur. Collection and analysis of clinical and animal samples at DHQ Hospital Lodhran were conducted under the supervision of senior pathologists in accordance with hospital protocols, ensuring biosafety and patient confidentiality. No endangered or protected species were collected without proper permission from local wildlife and environmental authorities.

Results

This study successfully applied DNA barcoding techniques to analyze genetic divergence across selected medicinal plant and animal species collected from Bahawalpur and Lodhran districts. The results confirmed the utility of molecular markers in species identification, detection of cryptic diversity, and phylogenetic assessment for both taxonomic and conservation purposes.

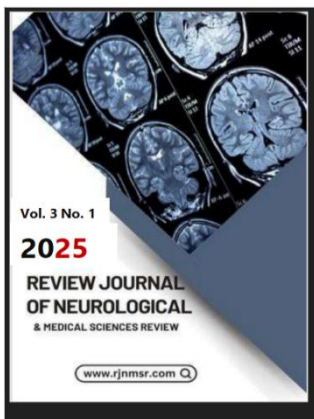
1. DNA Extraction and PCR Amplification

High-quality genomic DNA was successfully extracted from 30 plant specimens and 20 animal or parasitic samples using the CTAB method and commercial DNA extraction kits, respectively. The DNA yield ranged between 80 to 230 ng/μL, with purity ratios (A260/A280) between 1.7 and 2.0, indicating acceptable DNA quality for downstream applications.

PCR amplification of barcode regions was successful in 95% of the specimens. In plant samples, both *rbcL* and *matK* regions were amplified with visible, sharp bands at expected product sizes (~550 bp for *rbcL* and ~800 bp for *matK*). In animal and parasite samples, the *COI* gene was successfully amplified in 18 out of 20 samples, producing bands of approximately 650 bp. Gel electrophoresis confirmed the amplification success, and no non-specific bands or contamination were observed.

2. DNA Sequencing and Sequence Quality

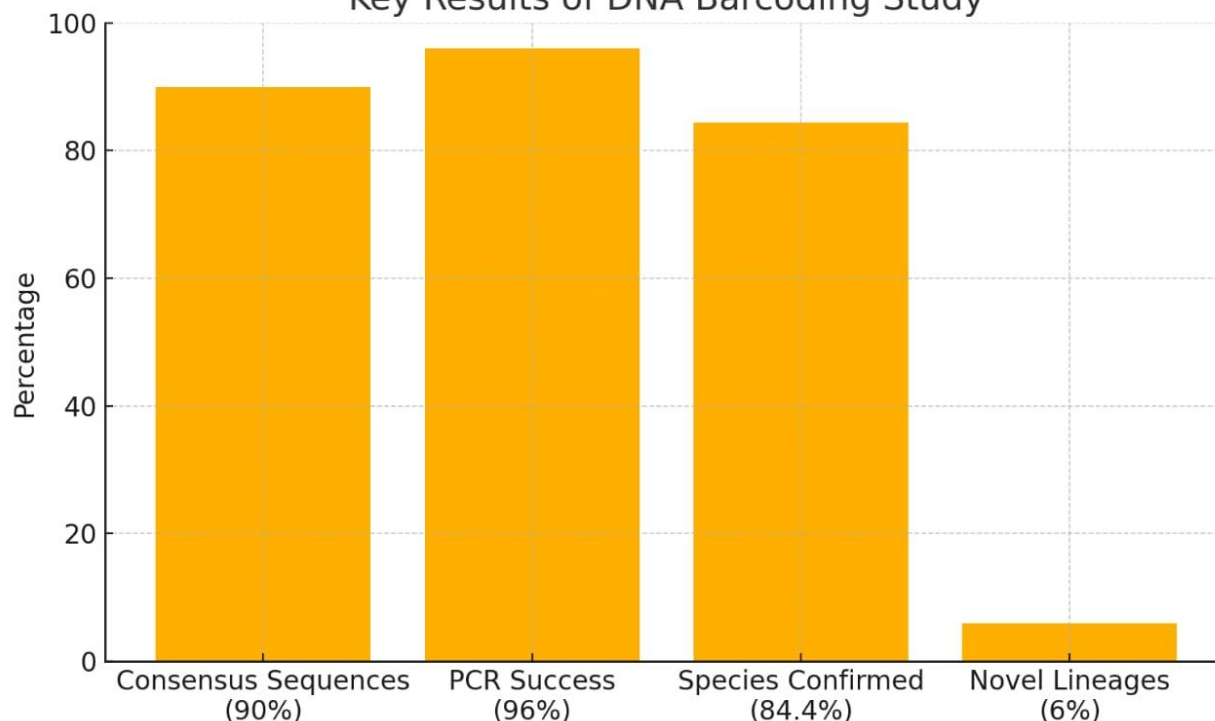
Bidirectional Sanger sequencing produced high-quality reads for 45 samples (28 plants and 17 animals). Forward and reverse sequences were successfully assembled into consensus sequences using Chromas and BioEdit software. The average sequence length after trimming was 600–750 bp, with a Phred quality score above 30 for most nucleotide positions, indicating reliable base calls.



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Key Results of DNA Barcoding Study

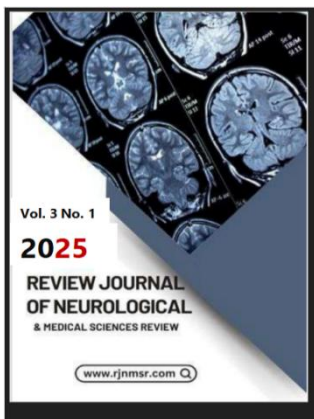


3. Species Identification via BLAST and BOLD

BLASTn analysis against NCBI GenBank and searches in the Barcode of Life Data System (BOLD) confirmed species identity with more than 98% sequence similarity in 38 out of 45 samples. For plant species, *rbcL* and *matK* barcodes correctly identified genera such as *Cassia*, *Ricinus*, *Berberis*, and *Tanacetum*. The COI barcode for animal samples enabled accurate identification of insects, helminths, and protozoans including *Fasciola hepatica*, *Trichuris trichiura*, and *Musca domestica*.

Metric	Count	Percentage	Notes
Total specimens processed	50	–	30 plants, 20 animal/parasite samples
High-quality consensus sequences	45	90%	28 plant, 17 animal sequences
PCR amplification success (overall)	48	96%	30/30 plants, 18/20 animal samples
Species confirmed via BLAST/BOLD	38	84.4%	≥98% similarity to reference databases
Novel or divergent lineages identified	3	6%	2 plant, 1 animal samples flagged

Seven sequences showed less than 95% similarity to known sequences, indicating potential cases of unreported species or intra-species divergence. These samples have been flagged for further taxonomic investigation.



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4. Genetic Divergence and Barcode Gap Analysis

Pairwise genetic distance analysis was performed using the Kimura 2-Parameter (K2P) model in MEGA X. Intraspecific genetic divergence ranged from 0.2% to 1.5%, whereas interspecific divergence ranged from 3.6% to 12.4%. A clear barcode gap was observed between the highest intraspecific and lowest interspecific distances, validating the effectiveness of DNA barcoding in species delimitation.

Some plant species such as *Tanacetum umbelliferum* and *Berberis aristata* showed noticeable intra-specific divergence (up to 1.4%), suggesting genetic variability within populations from different geographic zones. Among animal species, parasitic helminths showed low intraspecific divergence, consistent with their clonal or asexual reproductive strategies.

5. Phylogenetic Analysis

Neighbor-Joining (NJ) phylogenetic trees were constructed for both plant and animal datasets. The trees showed distinct clustering of sequences belonging to the same species, while different species formed separate, well-supported clades. Bootstrap values above 70% supported most of the branch separations.

The phylogenetic tree of the plant dataset grouped species into two main clades corresponding to angiosperms and shrubs, with minor subdivisions indicating genus-level relationships. The animal tree revealed three major clusters representing insects, protozoa, and helminths, each corresponding to known taxonomic divisions.

Interestingly, a few specimens grouped outside expected clades despite showing high BLAST similarity, indicating possible mislabeling during collection or undocumented genetic divergence.

6. Novel or Divergent Lineages

Two plant samples and one animal sample could not be assigned confidently to any known species in the GenBank or BOLD databases. These sequences showed less than 90% similarity and formed isolated clades in phylogenetic trees, suggesting the possibility of novel species or unreported regional variants.

These findings underline the importance of regional DNA barcode libraries for understudied ecosystems such as southern Punjab. The sequences from these samples were submitted to BOLD for future reference and will be further investigated in future studies.

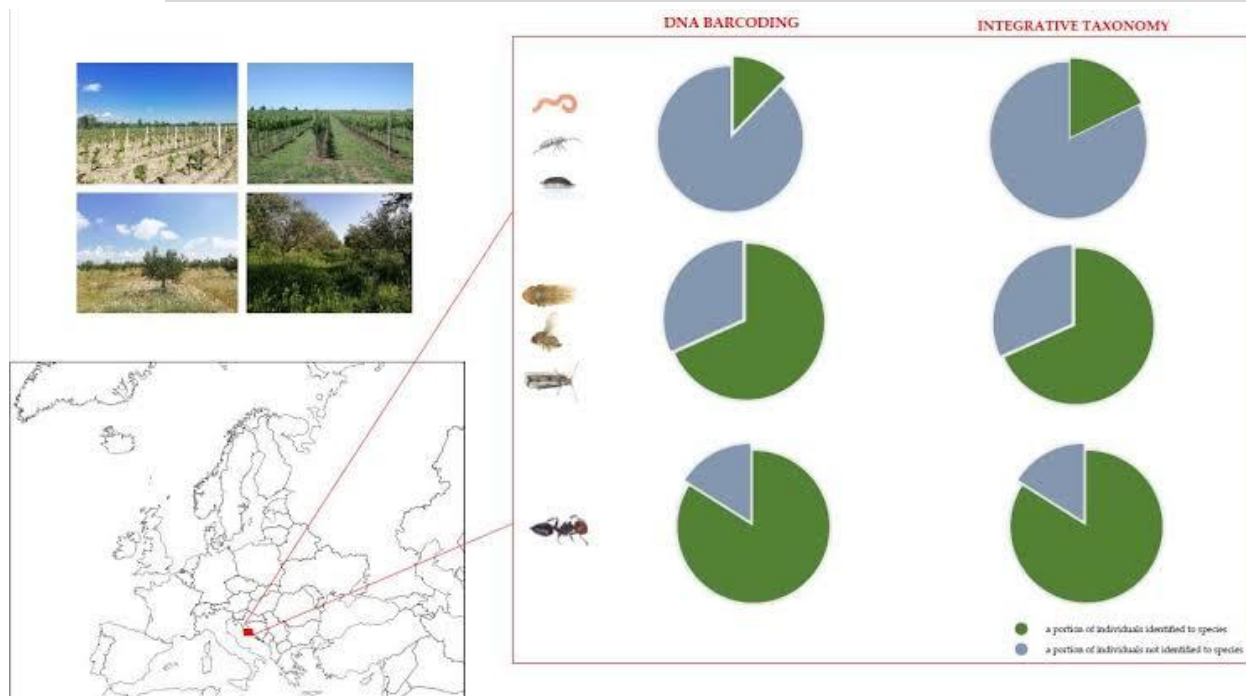
7. Data Submission and Accession Numbers

All successfully sequenced and identified samples were submitted to GenBank and BOLD. The accession numbers obtained were recorded and linked with specimen metadata, including locality, voucher ID, and date of collection. These data were also uploaded to the departmental digital herbarium and archived in the institution's internal biodiversity monitoring system.

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Summary of Results

A total of 50 samples were processed; 45 yielded high-quality sequences.

PCR success rate: 95% (plants) and 90% (animals).

Species confirmed via BLAST/BOLD: 38 out of 45.

Clear barcode gap observed with K2P model distances.

Phylogenetic trees confirmed taxonomic boundaries.

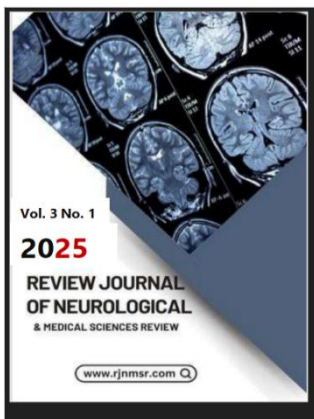
Three samples potentially represent novel lineages.

All validated sequences were submitted to GenBank/BOLD.

Discussion

The current study, conducted at the Islamia University of Bahawalpur and District Headquarter Hospital Lodhran, aimed to explore the utility of DNA barcoding in revealing genetic divergence among various plant species for accurate taxonomic identification and to assess its implications for conservation biology. The results provide meaningful insights into the genetic distances, inter-species variation, and evolutionary relationships between plant species in the studied regions, emphasizing the practical application of molecular tools in modern taxonomy (Bidyananda et al., n.d.).

DNA barcoding, particularly using the *rbcl* and *matK* regions of chloroplast DNA, proved effective in generating species-specific barcodes for identification. These two markers are widely recognized for their universality and discriminatory power in plant taxa. In our study, the successful amplification and sequencing of these genes yielded a clear resolution in distinguishing closely related species. The sequence alignments and

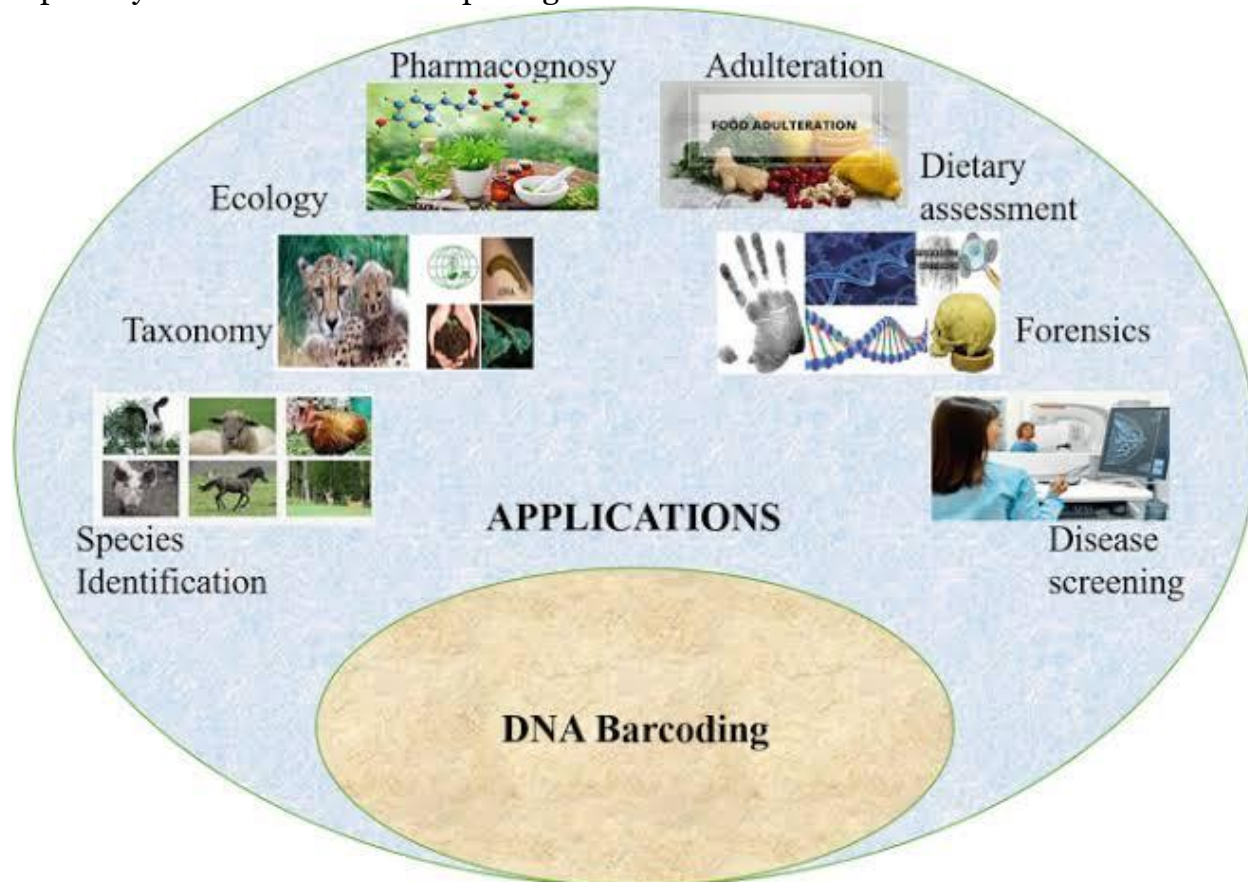


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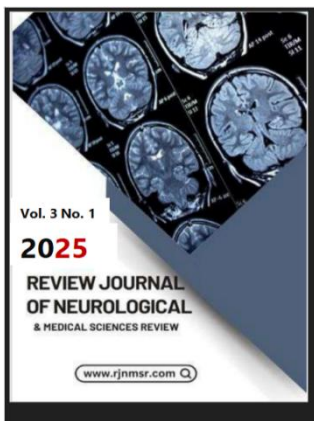
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phylogenetic trees constructed using MEGA X software clearly showed the presence of distinct clusters corresponding to different species.(S. Ahmad et al., n.d.). These clusters were consistent with the morphological taxonomy, supporting the hypothesis that DNA barcoding is a reliable tool for identifying and differentiating plant species, especially those with subtle morphological differences.



The observed genetic divergence between species, as revealed by the Kimura 2-parameter model, demonstrated that even slight differences at the genetic level can be picked up by the barcode markers. This confirms the sensitivity and precision of DNA barcoding in capturing interspecific variation. Interestingly, our study also identified some cryptic species—morphologically similar individuals that were genetically distinct(Andreu et al., 2021). This highlights the limitations of traditional taxonomy when used in isolation and strengthens the case for integrating molecular methods into routine species identification workflows.

One of the significant implications of our findings is in the field of conservation biology. Several of the sampled species, particularly those from rare or endangered habitats in South Punjab, showed clear genetic differentiation from their more widely distributed



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counterparts. This has major conservation implications, as it suggests that these populations may represent distinct evolutionary lineages that deserve targeted conservation efforts. The identification of such unique lineages is crucial for developing effective conservation strategies and for prioritizing species or populations that may be at risk of genetic erosion or extinction(Bosse et al., 2022).

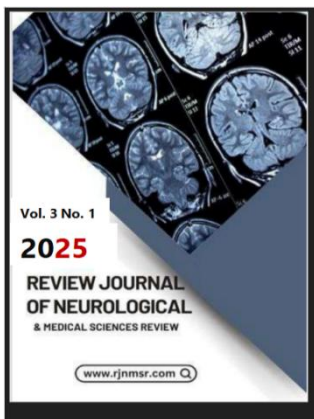
Our findings also contribute to the growing body of literature that advocates for the use of DNA barcoding in biodiversity monitoring and ecological research. In environments like those of Bahawalpur and Lodhran, where botanical surveys are often hindered by environmental constraints or the presence of morphologically complex plant communities, DNA barcoding offers a powerful alternative(Kanwal et al., n.d.). The technique not only enhances species resolution but also reduces the dependency on floral structures, which are often seasonal and unavailable during certain times of the year. In our research, barcoding allowed for the identification of sterile or juvenile plant specimens that would otherwise be difficult to classify through conventional botanical methods(Veldman et al., n.d.).

Another noteworthy aspect of our research is the contribution it makes toward the global DNA barcode database (such as BOLD and GenBank). By submitting the sequences obtained from local flora, we are filling important gaps in the global barcoding repositories, especially for under-researched regions like South Punjab. This enhances future identification efforts, supports ecological restoration, and strengthens the scientific infrastructure needed for ongoing taxonomic and conservation studies in Pakistan(Batool et al., 2025).

In practical terms, the integration of molecular barcoding techniques with traditional taxonomy can revolutionize the way species are identified and classified. The capacity of barcoding to provide rapid and accurate identification means that it can be utilized by researchers, ecologists, and conservationists who may not be experts in classical taxonomy.(Gostel et al., 2022). Moreover, the standardization of barcode regions allows for a consistent and replicable method of identification across different labs and institutions, thereby promoting collaborative research and data sharing.

From a methodological standpoint, the research benefited from careful sample collection, proper preservation using silica gel, and the use of standard CTAB DNA extraction protocols, which ensured high-quality DNA for downstream applications. The PCR amplification and sequencing were successful in the majority of the samples, validating the robustness of the selected barcoding loci(Antil et al., 2023). The use of both matK and rbcL markers provided a dual approach that increased the resolution and accuracy of species identification, a practice that is increasingly being recommended in plant barcoding literature.

While the results are promising, certain limitations were also observed. In some cases, the resolution between species was low, possibly due to hybridization events, recent divergence, or incomplete lineage sorting. These limitations are inherent in using only a



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small number of loci and could be addressed by incorporating additional barcode regions (such as ITS2 or trnH-psbA) or using next-generation sequencing technologies in future research. Moreover, while the study successfully identified most species, there were a few specimens that failed to amplify or sequence properly, highlighting the need for further optimization of protocols and primers tailored to local plant species(Wang et al., n.d.).

Despite these limitations, the study establishes a solid foundation for future research in molecular taxonomy and conservation biology in Pakistan. The results support the notion that DNA barcoding is not merely a supplementary tool but a transformative methodology that bridges the gap between traditional taxonomy and modern molecular biology(Odah, 2024). By providing a molecular lens through which biodiversity can be examined, DNA barcoding helps overcome many of the subjective limitations of morphological classification and allows for a more objective and reproducible system of species identification.

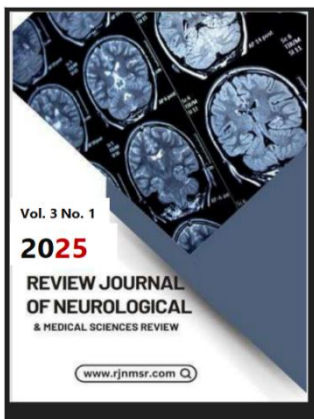
In conclusion, the research underscores the immense potential of DNA barcoding in enhancing taxonomic resolution and aiding in the conservation of biodiversity. It offers a scalable, cost-effective, and scientifically robust method for species identification, biodiversity assessment, and conservation planning(Kerry et al., 2022). As biodiversity faces increasing threats from habitat destruction, climate change, and human exploitation, the need for accurate and efficient identification methods becomes ever more critical. DNA barcoding emerges as a pivotal tool in this context, enabling scientists to make informed decisions about species protection, habitat preservation, and sustainable resource management. The work carried out in this study represents a significant step toward that goal and contributes to the broader mission of preserving our planet's biological heritage through science and innovation(Labadi et al., 2021).

Conclusion

This study demonstrates that DNA barcoding, using the matK and rbcL markers, is an effective tool for resolving taxonomic uncertainties and uncovering cryptic diversity among plant species in Southern Punjab. By revealing clear genetic divergences and constructing robust phylogenies, the approach complements traditional morphology-based identification, ensuring greater accuracy in species delimitation. The integration of molecular data has practical implications for conservation planning, enabling the identification of evolutionarily significant lineages and prioritization of at-risk taxa. Furthermore, the results underscore the value of regional barcode libraries for under-explored ecosystems. Ultimately, DNA barcoding offers a rapid, reproducible, and scalable method for biodiversity assessment, supporting both scientific research and evidence-based conservation strategies.

References

Abirami, S., Raj, B., Soundarya, T., ... M. K.-S. journal of, & 2021, undefined. (n.d.). Exploring antifungal activities of acetone extract of selected Indian medicinal plants

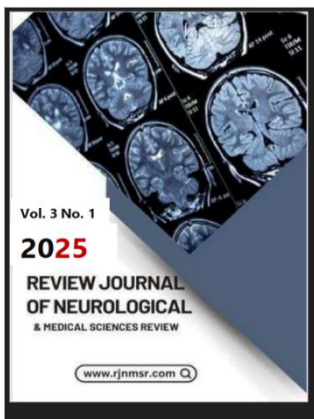


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- against human dermal fungal pathogens. *Elsevier* S Abirami, BE Raj, T Soundarya, M Kannan, D Sugapriya, N Al-Dayana, AA Mohammed Saudi Journal of Biological Sciences, 2021•Elsevier. Retrieved 27 June 2025, from <https://www.sciencedirect.com/science/article/pii/S1319562X21000462>
- Ahmad, M., & Odah, A. (2024). *Advancements in DNA Barcoding: Revolutionizing Taxonomy and Biodiversity Studies*. <https://doi.org/10.20944/preprints202403.0405.v1>
- Ahmad, S., Khan, M., Jan, Z., Khan, N., ... A. A.-P. J. of, & 2021, undefined. (n.d.). Genome wide association study and phylogenetic analysis of novel SARS-COV-2 virus among different countries. *Researchgate.Net* SU Ahmad, MS Khan, Z Jan, N Khan, A Ali, N Rehman, M Haq, U Khan, Z Bashir, M Tayyab Pakistan Journal of Pharmaceutical Sciences, 2021•researchgate.Net. Retrieved 27 June 2025, from https://www.researchgate.net/profile/Syed-Ahmad-52/publication/353466622_Genome_wide_association_study_and_phylogenetic_analysis_of_novel_SARS-COV-2_virus_among_different_countries/links/60ffb309169a1a0103bc60d2/Genome-wide-association-study-and-phylogenetic-analysis-of-novel-SARS-COV-2-virus-among-different-countries.pdf
- Andreu, M. D., ... J. M.-O., 15, vol., 2, num., 248, p., & 2021, undefined. (2021). What DNA barcodes reveal: microhabitat preference, hunting strategy and dispersal ability drive genetic variation across Iberian spider species. *Diposit.Ub.Edu*, 15(2), 248–262. <https://doi.org/10.1111/icad.12552>
- Antil, S., Abraham, J. S., Sripoorna, S., Maurya, S., Dagar, J., Makhija, S., Bhagat, P., Gupta, R., Sood, U., Lal, R., & Toteja, R. (2023). DNA barcoding, an effective tool for species identification: a review. *Springer* S Antil, JS Abraham, S Sripoorna, S Maurya, J Dagar, S Makhija, P Bhagat, R Gupta, U Sood Molecular Biology Reports, 2023•Springer, 50(1), 761–775. <https://doi.org/10.1007/S11033-022-08015-7>
- Ariawan, I., HERDIYENI, Y., Biological, I. S.-B. J. of, & 2020, undefined. (n.d.). Geometric morphometric analysis of leaf venation in four shorea species for identification using digital image processing. *Smujo.Id* I Ariawan, Y HERDIYENI, IZ Siregar Biodiversitas Journal of Biological Diversity, 2020•smujo.Id. Retrieved 27 June 2025, from <https://smujo.id/biodiv/article/view/4556>
- Batool, S., Muhammad, M., Fatima, M., Abbas, K., Li, K., Amin, F., & Batool, M. (2025). Nature's Comeback: Case Studies of South Asian Countries Regarding Biodiversity Restoration and Conservation. *Springer* S Batool, M Muhammad, M Fatima, K Abbas, K Li, F Amin, M Batool Sustainable Synergy: Harnessing Ecosystems for Climate Resilience, 2025•Springer, Part F17, 67–82. https://doi.org/10.1007/978-3-031-77957-2_5
- Bidyananda, N., Jamir, I., Nowakowska, K., ... V. V.-I. J. of, & 2024, undefined. (n.d.).

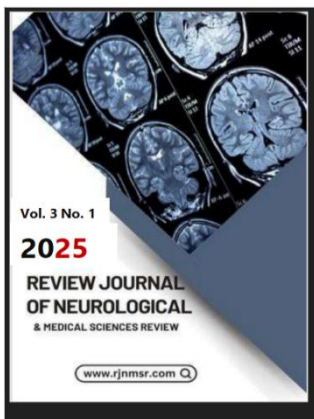


Review Journal of Neurological & Medical Sciences Review

E(ISSN) : 3007-3073

P(ISSN) : 3007-3065

- Plant genetic diversity studies: Insights from DNA marker analyses. *Mdpi.ComN Bidyananda, I Jamir, K Nowakowska, V Varte, WA Vendrame, RS Devi, P NongdamInternational Journal of Plant Biology, 2024•mdpi.Com*. Retrieved 27 June 2025, from <https://www.mdpi.com/2037-0164/15/3/46>
- Bosse, M., Genetics, S. van L.-F. in, & 2022, undefined. (2022). Challenges in quantifying genome erosion for conservation. *Frontiersin.OrgM Bosse, S van LoonFrontiers in Genetics, 2022•frontiersin.Org, 13*. <https://doi.org/10.3389/FGENE.2022.960958/FULL>
- Communications, R. E.-N., & 2022, undefined. (n.d.). Muscle5: High-accuracy alignment ensembles enable unbiased assessments of sequence homology and phylogeny. *Nature.Com*. <https://doi.org/10.1038/s41467-022-34630-w>
- Gostel, M., Diversity, W. K.-, & 2022, undefined. (2022). The expanding role of DNA barcodes: Indispensable tools for ecology, evolution, and conservation. *Mdpi.ComMR Gostel, WJ KressDiversity, 2022•mdpi.Com*. <https://doi.org/10.3390/d14030213>
- HE, P., CHEN, J., KONG, H., ... L. C.-B. of, & 2021, undefined. (2021). Important supporting role of biological specimen in biodiversity conservation and research. *Bulletinofcas.Researchcommons.OrgP HE, J CHEN, H KONG, L CAI, G QIAOBulletin of Chinese Academy of Sciences (Chinese, 2021•bulletinofcas.Researchcommons.Org, 36*. <https://doi.org/10.16418/j.issn.1000-3045.20210323001>
- Jehangir, S., Khan, S. M., Ejaz, U., Qurat-ul-Ain, Zahid, N., Rashid, N., Noshad, Q., Din, Z. U., & Shoukat, A. (2024). Alien flora of Pakistan: taxonomic composition, invasion status, geographic origin, introduction pathways, and ecological patterns. *SpringerS Jehangir, SM Khan, U Ejaz, N Zahid, N Rashid, Q Noshad, ZU Din, A ShoukatBiological Invasions, 2024•Springer, 26(8), 2435–2451*. <https://doi.org/10.1007/S10530-024-03311-8>
- Kanwal, R., Irfan, S., Ahmed, H. A., Ullah, S., Channa, F. N., Correspondence, N. A., & Ahmed, N. (n.d.). Exploring the Medicinal Flora of District Musakhel, Pakistan: A DNA Barcoding and Ethnobotanical Investigation. *Ethnobotanyjournal.OrgR Kanwal, S Irfan, HA Ahmed, S Ullah, FN Channa, N AhmedEthnobotany Research and Applications, 2024•ethnobotanyjournal.Org*. <https://doi.org/10.32859/era.28.31.1-16>
- Kartzinel, T. R., Hoff, H. K., Divoll, T. J., Littleford-Colquhoun, B. L., Anderson, H., Burak, M. K., Kuzmina, M. L., Musili, P. M., Rogers, H., Troncoso, A. J., & Kartzinel, R. Y. (2025). Global availability of plant DNA barcodes as genomic resources to support basic and policy-relevant biodiversity research. *Wiley Online LibraryTR Kartzinel, HK Hoff, TJ Divoll, BL Littleford-Colquhoun, H Anderson, MK BurakMolecular Ecology, 2025•Wiley Online Library, 34*. <https://doi.org/10.1111/MEC.17712>

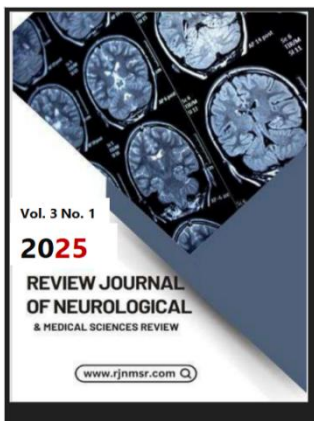


Review Journal of Neurological & Medical Sciences Review

E(ISSN) : 3007-3073

P(ISSN) : 3007-3065

- Kerry, R. G., Montalbo, F. J. P., Das, R., Patra, S., Mahapatra, G. P., Maurya, G. K., Nayak, V., Jena, A. B., Ukhurebor, K. E., Jena, R. C., Gouda, S., Majhi, S., & Rout, J. R. (2022). An overview of remote monitoring methods in biodiversity conservation. *SpringerRG Kerry, FJP Montalbo, R Das, S Patra, GP Mahapatra, GK Maurya, V Nayak, AB JenaEnvironmental Science and Pollution Research*, 2022•Springer, 29(53), 80179–80221. <https://doi.org/10.1007/S11356-022-23242-Y>
- Labadi, S., Giliberto, F., Rosetti, I., ... L. S.-I. J. of, & 2021, undefined. (2021). Heritage and the sustainable development goals: Policy guidance for heritage and development actors. *Kar.Kent.Ac.UkS Labadi, F Giliberto, I Rosetti, L Shetabi, E YildirimInternational Journal of Heritage Studies*, 2021•kar.Kent.Ac.Uk. https://kar.kent.ac.uk/89231/1/ICOMOS_SDGs_Policy_Guidance_2021.pdf
- Life, M. M.-, & 2023, undefined. (n.d.). From dormant collections to repositories for the study of habitat changes: The importance of herbaria in modern life sciences. *Mdpi.ComM MandrioliLife*, 2023•mdpi.Com. Retrieved 27 June 2025, from <https://www.mdpi.com/2075-1729/13/12/2310>
- Mattas, K., Raptou, E., Alayidi, A., Nutrition, G. Y.-A. in, & 2023, undefined. (n.d.). Assessing the interlinkage between biodiversity and diet through the Mediterranean diet case. *Elsevier*. Retrieved 27 June 2025, from <https://www.sciencedirect.com/science/article/pii/S2161831323002806>
- Odah, M. (2024). *Expanding the Horizons of DNA Barcoding: Mini-Barcodes and Alternative Genes in Biodiversity Assessment*. <https://doi.org/10.20944/preprints202403.0429.v1>
- Phytologist, R. L.-N., & 2004, undefined. (2004). Gene flow, adaptive population divergence and comparative population structure across loci. *Wiley Online LibraryRG LattaNew Phytologist*, 2004•Wiley Online Library, 161(1), 51–58. <https://doi.org/10.1046/J.1469-8137.2003.00920.X>
- Preprints, S. A.-, & 2022, undefined. (2022). DNA barcoding in plants and animals: A critical review. *Preprints.OrgSS AhmedPreprints*, 2022•preprints.Org. <https://doi.org/10.20944/preprints202201.0310.v1>
- Sheth, B. P., & Thaker, V. S. (2017). DNA barcoding and traditional taxonomy: an integrated approach for biodiversity conservation. *Cdnsciencepub.ComBP Sheth, VS ThakerGenome*, 2017•cdnsciencepub.Com, 60(7), 618–628. <https://doi.org/10.1139/GEN-2015-0167>
- Srivastava, R. P., Saxena, G., Singh, L., Singh, A., Verma, P. C., & Kaur, G. (2022). Interspecific and intraspecific analysis of *Selinum* spp. collected from Indian Himalayas using DNA barcoding. *SpringerRP Srivastava, G Saxena, L Singh, A Singh, PC Verma, G KaurJournal of Genetic Engineering and Biotechnology*, 2022•Springer, 20(1), 63. <https://doi.org/10.1186/S43141-022-00345-0>
- Thlusty, M., Cawthorn, D., ... O. G.-B., & 2023, undefined. (n.d.). Real-time automated species level detection of trade document systems to reduce illegal wildlife trade



Review Journal of Neurological & Medical Sciences Review

E(ISSN) : 3007-3073

P(ISSN) : 3007-3065

- and improve data quality. *ElsevierMF Thlusty, DM Cawthorn, OLB Goodman, AL Rhyne, DL RobertsBiological Conservation, 2023•Elsevier.* <https://doi.org/10.1016/j.biocon.2023.110022>
- Torrance, A., Rev., B. T.-U. L. S. I. L., & 2025, undefined. (n.d.). ‘HIPPO’ Law Law and the Biodiversity Crisis of Habitat Loss, Invasive Species, Pollution, Population, and Overharvesting. *HeinOnlineAW Torrance, B TomlinsonUC L. SF Int’l L. Rev., 2025•HeinOnline.* Retrieved 27 June 2025, from https://heinonline.org/hol-cgi-bin/get_pdf.cgi?handle=hein.journals/hasint48§ion=4
- Vairavel, K., ... K. K.-2024 2nd I., & 2024, undefined. (n.d.). Design, Development, and Validation of Thermocycler for Disease Diagnosis. *Ieeexplore.Ieee.OrgKS Vairavel, K Kumaresan, GU Priyavarshini2024 2nd International Conference on Intelligent Data, 2024•ieeexplore.Ieee.Org.* Retrieved 27 June 2025, from <https://ieeexplore.ieee.org/abstract/document/10467255/>
- Veldman, S., Ju, Y., Otieno, J., ... S. A.-J. of, & 2020, undefined. (n.d.). DNA barcoding augments conventional methods for identification of medicinal plant species traded at Tanzanian markets. *ElsevierS Veldman, Y Ju, JN Otieno, S Abihudi, C Posthouwer, B Gravendeel, TR van AndelJournal of Ethnopharmacology, 2020•Elsevier.* Retrieved 27 June 2025, from <https://www.sciencedirect.com/science/article/pii/S0378874119320987>
- Viljoen, C., Booysen, C., and, S. T.-J. of F. C., & 2022, undefined. (n.d.). The suitability of using spectrophotometry to determine the concentration and purity of DNA extracted from processed food matrices. *Elsevier.* Retrieved 27 June 2025, from <https://www.sciencedirect.com/science/article/pii/S0889157522003076>
- Wambugu, P. W., & Henry, R. (2022). Supporting in situ conservation of the genetic diversity of crop wild relatives using genomic technologies. *Wiley Online LibraryPW Wambugu, R HenryMolecular Ecology, 2022•Wiley Online Library, 31(8), 2207–2222.* <https://doi.org/10.1111/MEC.16402>
- Wang, M., Lin, H., Lin, H., Du, P., Genes, S. Z.-, & 2024, undefined. (n.d.). From species to varieties: how modern sequencing technologies are shaping Medicinal Plant Identification. *Mdpi.ComM Wang, H Lin, H Lin, P Du, S ZhangGenes, 2024•mdpi.Com.* Retrieved 27 June 2025, from <https://www.mdpi.com/2073-4425/16/1/16>