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Popular Article

Scope of DNA Barcoding in Livestock Improvement

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INTRODUCTION

In forensic investigations concerning animal abuse and biodiversity preservation, DNA analysis is frequently employed. It can be used to look into crimes including poaching, illegal trade in protected species, habitat devastation, unlawful gathering, and maltreatment. Moreover, DNA analysis can be used to ascertain an animal's species, place of origin, and genetic relationships with other animals. For the purpose of genetic species identification, nuclear and mitochondrial markers can be employed.

Methods like gel electrophoresis, Single-Strand Conformational Polymorphism DNA barcoding, isotope analysis, and mitochondrial microsatellite analysis Microscopy Ballistics, among other techniques, can be used to identify certain species and animals, as well as samples retrieved from crime scenes, illicit wildlife traffickers, and underground markets engaged in the trade in wildlife. Animal identification, in contrast to human identification, usually uses DNA-based investigations to identify an animal by determining its family, genus, species, and sex.

As the basis of all biology, the ability to identify, name, and classify living things at the species level has emerged as a crucial requirement for biodiversity study and management, conservation, and breeding. (Le and Vu, 2019) The majority of the time, one or a few important morphological characteristics are used to identify and classify species. DNA barcoding is one of the sophisticated molecular tools and procedures that have been used to uncover species complexity and find new ones due to the morphological complexity of the organisms.

WHAT IS DNA BARCODING?

It is a diagnostic method that identifies species within a group of organisms as well as unidentified species by using a brief DNA sequence or sequences. To identify a specific or unknown species, a brief DNA segment taken from a taxonomically unknown item is sequenced and compared with the previously available sequences in the reference library or databases (Wilson, 2012). These sequences are exclusive to that one species, making it easy to detect and calculate genetic variability within and between species (Krishnamurthy and Francis, 2012).

Although the livestock industry generates significant economic benefits, there are issues with quality control. Barcoding technology is a key tool for promoting high-quality livestock food and reducing food piracy, benefiting both public and animal health (Barcaccia et al., 2016).The early DNA sequencing study on microbial communities utilising the 5S rRNA gene led to the development of DNA barcoding techniques (Woese et al., 1990). In a 2003 paper, Paul D.N. Hebert et al. from the University of Guelph, Ontario, Canada, proposed specific techniques and terminology of modern DNA barcoding as a standardised method for identifying species, as well as potentially allocating unknown sequences to higher taxa such as orders and phyla (Hebert et al., 2003). Hebert and his associates found that the majority of animal species could be identified using a brief DNA sequence taken from the mitochondria. Because of his work creating a genetic barcode to categorise all living things on Earth, Hebert is referred to as the "father of DNA barcoding". Hebert and associates provided evidence on the function of the cytochrome c oxidase I (COI) gene.

WHY BARCODING?

1. **Works for all stages of life** – Barcoding can be applied to all stage of life, from birth to death, to track and manage various aspects of an individual's life.
2. **Works with fragments** - Barcoding allows researchers to extract valuable information from limited or degraded samples, advancing our understanding of biology, evolution, and disease.
3. **Unmasks look-alikes** - Means using a unique barcode or identifier to reveal or identify something that was previously hidden or anonymous.
4. **Reduce ambiguity** - By reducing ambiguity, barcoding increases clarity, accuracy, and efficiency in various applications, making it an essential tool in many industries.
5. **Expertise to go further** - It's about taking barcoding to the next level and unlocking its full potential.
6. **Democratize access** - Means making information, resources, or benefits more widely available and accessible to a larger audience, regardless of their background, location, status, through the use of barcodes.
7. **Demonstrates the value of collection** - Means that the use of barcodes showcases the importance and benefits of collecting and managing data, items, or resources in a systematic and organized way.
8. **Speed writing the life of the encyclopedia** - Rapidly documenting the history and evolution of encyclopedias.

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CRITERIA FOR GENE REGION

For a gene area to be used as a useful DNA barcode, it must meet three requirements: (i) Have considerable genetic divergence and variability at the species level. (ii) Have conserved flanking sites that can be used to create universal PCR primers with a broad taxonomic range. (iii) Have a short sequence length to support DNA extraction and amplification techniques currently in use.

Prokaryotes	RNA genes used for species identification
Animals, fishes, Birds	Small regions of the mitochondrial COI gene
Plants	rbcl gene sequence and matk gene sequence of chloroplast
Fungi	ITS region

For animal, 648-bp fragment of mitochondrial gene COI (cytochrome c oxidase subunit 1) has been chosen as standard barcoding marker (Hebert et.al, 2003). Hebert and his group declared mtDNA as a universal genetic barcode due to its low genetic recombination and maternally acquired haploid gene, making it useful for sequencing heterozygous organisms (Swartz et al., 2008). The COI region evolves at a faster rate than the nuclear genome, making it unique to each species. Using COI allows for discrimination against closely related species with low failure rates (Waugh, 2007).

METHODS INVOLVED IN DNA BARCODING

1. **Sample collection** - Barcoding can be done from tissue of a target specimen, a mixture of species (bulk sample), or DNA contained in environmental samples (e.g., water or soil).
2. **DNA extraction** - DNA barcoding relies on pure, high-quality DNA obtained from various sources under sterilised settings. To extract DNA from biological samples with low DNA content, thorough sampling and homogenisation are necessary (Yang et al., 2018).
3. **PCR amplification** - The purified DNA sample's barcoding locus is amplified using Polymerase Chain Reaction (PCR) with specified primers that anneal the target DNA at a predetermined temperature. Choosing the right primer set is crucial for improving the sensitivity and efficiency of PCR for barcoding regions (Yang et al., 2018).
4. **DNA sequencing** - After amplifying the DNA barcode marker area, the next step is to sequence it using DNA sequencing tools (D'Amore et al., 2016). The Sanger sequencing approach is effective for small-scale sequencing of DNA barcodes from a single species. Parveen et al. (2016). NGS technology can successfully sequence numerous barcoding regions from mixed DNA samples, including environmental materials, culinary products, and pharmaceutical formulations.
5. **Sequence analysis** - Sequence analysis was performed using both online and offline bioinformatic techniques. Sequence analysis aims to accurately identify taxons by comparing novel sequences generated through barcoding research to database sequences.

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6. **Data analysis & interpretation** - Comparative analysis involves numerous methodologies, including similarity-, distance-, and tree-based approaches that allow the researcher to find variances in the sequences. The Basic Local Alignment Search Tool (BLAST) is an effective approach for finding similarity between query sequences and database sequences. Bioinformatic tools like MEGA, PHYLIP, and PAUP can create and visualise phylogenetic trees using hierarchical clustering algorithms like NJ, ML, MP, and BI (Hebert et al., 2003b; Elias et al., 2007).
7. **Species identification** - The taxonomic attribution of OTUs to species is accomplished by comparing sequences to reference libraries. The Basic Local Alignment Search Tool (BLAST) is a popular tool for identifying regions of similarity between sequences by comparing sequence readings from the sample to sequences in reference databases. (Alonso-Alemany et al., 2011) If the reference database contains sequences from the relevant species, the sample sequences can be recognised at the species level. If a sequence does not match an existing reference library entry, DNA barcoding can be utilised to build a new record.
8. **Documentation**- documentation" refers to the process of recording and maintaining accurate information about: Barcode labels, Items or products, Scanning and tracking, Records of quality control checks, verification processes, and any issues or discrepancies found.

TYPES OF DNA BARCODING

I. **Metabarcoding**

II. **Minibarcoding**

I. **Metabarcoding -** Meta barcoding is described as the barcoding of DNA or eDNA (environmental DNA) that enables the simultaneous identification of many taxa within the same sample, usually within the same organism group. The primary distinction between the methodologies is that metabarcoding, unlike barcoding, does not focus on a single organism but rather seeks to ascertain species composition within a sample.

Meta-barcoding identifies species-specific sequences from a combination of DNA amplified with a universal barcode. This can result in misleading sequencing results by creating several or overlapping sequence peaks (Parveen et al., 2016). Meta-barcoding can help identify several species in complex materials utilising Next Generation Sequencing and mitochondrial 16S ribosomal RNA. The NGS is a very efficient multitaxa identification method that distinguishes species-specific sequences from a combination of sequences as well as degraded DNA samples (Abubakar et al., 2017). This approach is effective for biodiversity research, ecological management, community analysis (Leray and Knowlton, 2015), and estimating cattle diet (Lee et al., 2018). Meta-barcoding has shown useful in identifying organisms in medicinal formulations, foods, and forensic materials (Raclariu et al., 2018).

II. **Minibarcoding –**

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It is a technique that uses A short fragment of DNA, often less than 200 bp, is being used for amplification (Meusnier et al., 2008). Shorter amplicons in mini-barcoding boost PCR efficiency, leading to higher success rates (Sarkinen et al., 2012). Mini-barcoding technology is faster and more efficient than regular barcoding as it uses a tiny section of DNA and particular primers to identify target taxons (Gao et al., 2019). Mini-barcoding technology can effectively amplify low-quality DNA from many sources where regular barcoding is inconvenient (de Boer et al., 2015).

STRENGTHS

- Enables easy species identification.
- Increases the discovery rate of new species.
- Adds data to reduce taxonomic uncertainty.
- Identifies species with less dna, saving time and money over previous approaches.
- Scalable procedures allow for objective species identification.
- Generates references for matching DNA of unknown origin, such as metagenomics and eDNA.

LIMITATIONS

- Database incompleteness - the barcode data must include the reference sequence for species identification.
- Technological bias exists, including the potential of DNA sample contamination by PCR inhibitors, primer bias, and so on.
- Lack of standardisation - There is no agreement on DNA preservation or extraction procedures, DNA marker and primer sets, or PCR protocols.
- Mismatches between conventional (morphological) and barcode-based identification. Estimate of richness diversity.
- There is more genetic variety inside a species than between species.
- Hybrid species cannot be identified since DNA barcoding only uses one gene region.
- Lack of a universal DNA barcode marker.
- In the event of illegal wildlife trading, species identification may not be adequate to determine nation of origin. Special equipment is required.

OPPORTUNITIES

- Can adapt to new technology.
- Assists in revealing global biodiversity.
- The biomonitoring process may be automated.
- Provides molecular context for historical specimens.
- Can increase public engagement with genetic technologies.
- Improves international access to DNA resources.

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- Contributes to a dependable library of life.
- Can estimate species diversity using complex and environmental samples.

APPLICATIONS OF DNA BARCODING

- Authentication of geographical origin (Perdices et al., 2008)
- Identification of new or endangered species (Wynen et al., 2009)
- Validation of food product (Barbuto et al., 2010)
- Corroboration of closely related or ambiguous species (Shirak et al., 2009)
- Ornamental species trade (Steinke et al., 2009)
- Study of species diversity in a location (Kartavtsev et al., 2008)
- Species Discrimination studies (Pereira et al., 2013)
- Identification of mislabelled food products (Filonzi et al., 2010)
- Identification of smoked food products (Smith et al., 2008)

CONCLUSION

DNA barcoding has arisen and established itself as an important technique for species identification and phylogenetic analysis. It has proven effective in the protection of endangered species, the identification of agricultural pests and disease vectors, the detection of product adulteration, and environmental sustainability. DNA barcoding efficacy is dependent on the selection of an appropriate piece of DNA. It is not a part of DNA taxonomy, nor is it a tool for phylogenetic reconstruction; rather, it allows sample specimens to be linked directly to existing voucher specimens and taxonomic information.

REFERENCES

Abubakar, B.M., Mohd Salleh, F., Shamsir Omar, M.S. and Wagiran, A. (2017). Review: DNA Barcoding and Chromatography Fingerprints for the Authentication of Botanicals in Herbal Medicinal Products. Evidence-based complementary and alternative medicine. https://doi.org/10.1155/2017/1352948

Alonso-Alemany, D., Clemente, J.C., Jansson, J. and Valiente, G. (2011). Taxonomic Assignment in Metagenomics with TANGO. EMBnet.journal. 17 (2), 16–20.

Barbuto, M., Galimberti, A., Ferri, E., Labra, M., Malandra, R., Galli, P. and Casiraghi, M. (2010). DNA Barcoding Reveals Fraudulent Substitutions in Shark Seafood Products: The Italian case of "palombo" (Mustelus spp.). Food Research International, 43(1), 376-381.

Barcaccia, G., Lucchin, M. and Cassandro, M. (2016). DNA Barcoding as a molecular tool to track down mislabelling and food piracy. Diversity, 8(1), 2, https://doi.org/10.3390/d8010002

D'Amore, R., Ijaz, U.Z., Schirmer, M., Kenny, J.G., Gregory, R., Darby, A.C., Shakya, M., Podar, M., Quince, C. and Hall, N. (2016). A comprehensive benchmarking study of protocols and sequencing platforms for 16S rRNA community profiling. BMC genomics, 17, 55. https://doi.org/10.1186/s12864-015-2194-9

de Boer, H.J., Ichim, M.C. and Newmaster, S.G. (2015). DNA Barcoding and Pharmacovigilance of Herbal Medicines. Drug safety, 38(7), 611–620. https://doi.org/10.1007/s40264-015-0306-8

Elias, M., Hill, R.I., Willmott, K.R., Dasmahapatra, K.K., Brower, A.V., Mallet, J. and Jiggins, C.D. (2007). Limited performance of DNA barcoding in a diverse community of tropical butterflies. Proceedings. Biological sciences, 274 (1627), 2881–2889. https://doi.org/10.1098/rspb.2007.1035

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Filonzi, L., Stefania, C., Marina, V., and Francesco, N.M. (2010). Molecular barcoding reveals mislabeling of commercial fish products in Italy. Food Research International, 43, 1383-1388.

Gao, Z., Liu, Y., Wang, X., Wei, X. and Han, J. (2019). DNA Mini-Barcoding: A Derived Barcoding Method for Herbal Molecular Identification. Frontiers in plant science, 10, 987. https://doi.org/10.3389/fpls.2019.00987

Hebert, P. D.N., Cywinska, A., Ball, S.L. and DeWaard, J.R. (2003a). Biological identifications through DNA barcodes. Proceedings. Royal Society of London. B series Biological Sciences, 270, 313–321.

Hebert, P.D.N., Ratnasingham, S. and deWaard, J.R. (2003b). Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings. Royal Society of London. B series Biological Sciences. 270, S96–S99.

Kartavtsev, Y. P., Sharina, S. N., Goto, T., Chichvarkhin, A. Y., Balanov, A. A., Vinnikov, K. A., Ivankov, V. N., and Hanzawa, N. (2008). Cytochrome oxidase 1 gene sequence analysis in six flatfish species (Teleostei, Pleuronectidae) of Far East Russia with inferences in phylogeny and taxonomy. Mitochondrial DNA, 19(6), 479–489.

Krishnamurthy, K.P. and Francis, R.A. (2012). A critical review on the utility of DNA barcoding in biodiversity conservation. Biodivers. Conserv. 21, 1901–1919.

Lee, T.R.C., Alemseged, Y. and Mitchell, A. (2018). Dropping Hints: Estimating the diets of livestock in rangelands using DNA metabarcoding of faeces. MBMG 2, 1–17. https://doi.org/10.3897/mbmg.2.22467.

Meusnier, I., Singer, G.A., Landry, J.F., Hickey, D.A., Hebert, P.D. and Hajibabaei, M. (2008). A universal DNA mini-barcode for biodiversity analysis. BMC Genom. 9, 214. https://doi.org/10.1186/1471-2164-9-214.

Parveen, I., Gafner, S., Techen, N., Murch, S.J. and Khan, I.A. (2016). DNA barcoding for the identification of botanicals in herbal medicine and dietary supplements: strengths and limitations. Planta Med. 82 (14), 1225–1235.

Perdices, A., Bohlen, J. and Doadrio, I. (2008). The molecular diversity of adriatic spined loaches (Teleostei, Cobitidae). Molecular phylogenetics and evolution, 46(1): 382–390.

Pereira, L. H., Hanner, R., Foresti, F. and Oliveira, C. (2013). Can DNA barcoding accurately discriminate megadiverse Neotropical freshwater fish fauna?. BMC genetics, 14, 1-14.

Raclariu, A.C., Heinrich, M., Ichim, M.C. and de Boer, H. (2018). Benefits and limitations of DNA barcoding and metabarcoding in herbal product authentication. Phytochem. Anal. 29 (2), 123–128.

Sarkinen, T., Staats, M., Richardson, J.E., Cowan, R.S. and Bakker, F.T. (2012). How to open the treasure chest? Optimising DNA extraction from herbarium specimens https://doi.org/10.1371/journal.pone.0043808.

Shirak, A., Cohen-Zinder, M., Barroso, R.M., Seroussi, E., Ron, M., and Hulata, G. (2009). DNA Barcoding of Israeli Indigenous and Introduced Cichlids. The Israeli Journal of Aquaculture - Bamidgeh, 61(2), 83-88.

Smith, P. J., Mcveagh, S.M. and Steinke, D. (2008). DNA barcoding for the identification of smoked fish products.Journal of Fish Biology , 72, 464–471.

Steinke, D., Zemlak, T. S., and Hebert, P. D. (2009). Barcoding nemo: DNA-based identifications for the ornamental fish trade. PloS one, 4(7), 6300.

Swartz, E.R., Mwale, M. and Hanner, R. (2008). A role for barcoding in the study of African fish diversity and conservation. S. Afr. J. Sci. 104 (4), 293–298.

Vu, H. and Le, L. (2019). Bioinformatics analysis on DNA barcode sequences for species identification: a review. Annu. Res. Rev. Biol. 34 (1), 1–12.

Waugh, J. (2007). DNA barcoding in animal species: progress, potential and pitfalls. Bioessays 29 (2), 188–197.

Wilson, J.J. (2012). DNA barcodes for insects. DNA barcodes: methods and protocols. In: John Kress, W., Erickson, D.L. (Eds.), Methods in Molecular Biology. p. 858. https://doi.org/10.1007/978-1-61779-591-6_3.

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Woese, C. R., Kandler, O. and Wheelis, M. L. (1990). Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proceedings of the National Academy of Sciences of the United States of America, 87(12), 4576–4579.

Wynen, L., Larson, H., Thorburn, D.C., Peverell, S., Morgan, D., Field, I. and Gibb, K. (2009). Mitochondrial DNA supports the identification of two endangered river sharks (Glyphis glyphis and Glyphis garricki) across northern Australia. Marine and Freshwater Research, 60 (6): 554-562.

Yang, F., Ding, F., Chen, H., He, M., Zhu, S., Ma, X., Jiang, L., and Li, H. (2018). DNA Barcoding for the Identification and Authentication of Animal Species in Traditional Medicine. Evidence-based complementary and alternative medicine. https://doi.org/10.1155/2018/5160254