UNIVERSIDAD AUTÓNOMA CHAPINGO

UNIDAD REGIONAL UNIVERSITARIA DE ZONAS ÁRIDAS

Maestría en Ciencias en Recursos Naturales y Medio Ambiente en Zonas Áridas

MUESTREO INVASIVO Y NO INVASIVO EN EL ANÁLISIS GENÉTICO Y SU USO EN LA DETERMINACIÓN DEL MICROBIOMA FECAL DE BORREGO BERBERISCO (*Ammotragus lervia*), BOVINOS Y CAPRINOS EN EL DESIERTO CHIHUAHUENSE, MÉXICO.

INVASIVE AND NON-INVASIVE SAMPLING IN GENETIC ANALYSIS AND ITS USE IN DETERMINING THE FECAL MICROBIOME OF BARBARY SHEEP (Ammotragus lervia), CATTLE, AND GOATS IN THE CHIHUAHUAN DESERT, MEXICO.

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Bermejillo, Durango, México. Mayo 2026.

APROBADA

MUESTREO INVASIVO Y NO INVASIVO EN EL ANÁLISIS GENÉTICO Y SU USO EN LA DETERMINACIÓN DEL MICROBIOMA FECAL DE BORREGO BERBERISCO (*Ammotragus lervia*), BOVINOS Y CAPRINOS EN EL DESIERTO CHIHUAHUENSE, MÉXICO.

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DEDICATORIA

A mi padre Heriberto Ramírez y mi madre María del Carmen García, por sus atenciones, dedicación, esfuerzo además de ser una guía y motivación. A mi amada esposa Keren García, por tu apoyo constante y ser la única persona que creyó en mi. A mis hijos Levi Ramírez y el futuro retoño. Por ser mi motivación cada día. A mis hermanos Jair y Gamaliel Ramírez, por considerarme en sus vidas y estar siempre en disposición.

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"Y en efecto, pregunta ahora a las bestias, y ellas te enseñarán; A las aves de los cielos, y ellas te lo mostrarán; O habla a la tierra, y ella te enseñará; Los peces del mar te lo declararán también. ¿Qué cosa de todas estas no entiende Que la mano del Señor la hizo? En su mano está el alma de todo viviente,
Y el hálito de todo el género humano".

- *Job 12:7-10*

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Primer autor del artículo científico “TheEvolution and Scope of Invasive and Non-Invasive Sampling in Terrestrial Mammal Population Genetics: Implications for the Comparability of He, Ho and Fis: A Scientometric Review” Conservation-MDPI. DOI https://doi.org/10.3390/conservation6020053.

Primer autor del artículo científico “La Rana Leopardo de Río Grande (Lithobates berlandieri, Anura: Ranidae), características y calidad del hábitat en un ejido de Parras, Coahuila, México”. TIP. Revista Especializada en Ciencias Quimico-Biologicas. DOI:10.22201/fesz.23958723e.2025.710.

MUESTREO INVASIVO Y NO INVASIVO EN EL ANÁLISIS GENÉTICO Y SU USO EN LA DETERMINACIÓN DEL MICROBIOMA FECAL DE BORREGO BERBERISCO (*Ammotragus lervia*), BOVINOS Y CAPRINOS EN EL DESIERTO CHIHUAHUENSE, MÉXICO.

Resumen

La presente investigación documental integra un análisis cienciométrico y una revisión sistemática, además de un estudio microbiológico metagenómico, enfocados en la genética de la conservación y la ecología microbiana de mamíferos terrestres silvestres. Los resultados evidencian una rápida expansión de la genética poblacional en las últimas décadas y una transición hacia metodologías no invasivas, que han ampliado la cobertura taxonómica y geográfica mientras reducen el manejo animal. Aunque el muestreo invasivo proporciona ADN de mayor integridad, los avances en control de calidad, amplificación y secuenciación han incrementado la confiabilidad de los métodos no invasivos. Las diferencias en Ho, He y Fis fueron moderadas al considerar especies y marcadores, destacando la importancia del control de calidad y la transparencia metodológica. También se identificaron sesgos regionales y desafíos asociados con la transición de microsatélites a SNP. Adicionalmente, se analizó el microbioma fecal del arruí (*Ammotragus lervia*), bovinos (*Bos taurus*) y cabras (*Capra aegagrus hircus*) en simpatría en el Cañón de Santa Elena. Mediante secuenciación del gen 16S rRNA y análisis en QIIME2 y DADA2, se observó una composición similar dominada por Firmicutes y Bacteroidota, sugiriendo convergencia microbiana. Sin embargo, la diversidad beta mostró diferencias significativas ($p < 0.05$), evidenciando divergencia asociada al hospedero. En conjunto, estos resultados resaltan la importancia de integrar enfoques éticos, metodologías estandarizadas y ciencia abierta bajo el enfoque One Health, concluyendo que la combinación de genética y microbioma fortalece la comprensión ecológica y el manejo sostenible de especies en ecosistemas áridos.

Palabras clave: Genética de la conservación, microbioma fecal, mamíferos terrestres, muestreo no invasivo, One Health.

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Abstract

This literature review combines a scientometric analysis and a systematic review, as well as a metagenomic microbiological study, focusing on conservation genetics and the microbial ecology of wild terrestrial mammals. The results demonstrate a rapid expansion of population genetics in recent decades and a shift toward non-invasive methodologies, which have broadened taxonomic and geographic coverage while reducing animal handling. Although invasive sampling provides DNA of higher integrity, advances in quality control, amplification, and sequencing have increased the reliability of non-invasive methods. Differences in Ho, He, and Fis were moderate when considering species and markers, highlighting the importance of quality control and methodological transparency. Regional biases and challenges associated with the transition from microsatellites to SNPs were also identified. In addition, the fecal microbiome of Barbary sheep (*Ammotragus lervia*), cattle (*Bos taurus*), and goats (*Capra aegagrus hircus*) living in sympatry in the Santa Elena Canyon was analyzed. Through 16S rRNA gene sequencing and analysis in QIIME2 and DADA2, a similar composition dominated by Firmicutes and Bacteroidota was observed, suggesting microbial convergence. However, beta diversity showed significant differences ($p < 0.05$), indicating host-associated divergence. Taken together, these results highlight the importance of integrating ethical approaches, standardized methodologies, and open science under the One Health framework, concluding that the combination of genetics and the microbiome strengthens ecological understanding and the sustainable management of species in arid ecosystems.

Keywords: Conservation genetics, fecal microbiome, terrestial mammals, non-invasive sampling, One Health.

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CAPITULO 1. INTRODUCCIÓN GENERAL

En los últimos años se han realizado diversos esfuerzos para el estudio de mamíferos y otros grupos biológicos, con el propósito de obtener muestras genéticas de los ejemplares (Zemanova, 2019). La colecta de este material constituye uno de los principales retos en el análisis poblacional, debido a la vulnerabilidad de las especies y a la reducción de su variabilidad genética, en gran medida ocasionada por factores antropogénicos (Schlaepfer et al., 2018). Por ello, resulta fundamental llevar a cabo evaluaciones génicas en la fauna silvestre (Stetz et al., 2011). En este contexto, es indispensable investigar a los cuadrúpedos terrestres mediante técnicas de muestreo que no comprometan su integridad y que contribuyan a la interpretación genética de sus poblaciones (Schilling et al., 2022).

Entre las estrategias utilizadas para el estudio de la fauna destacan el muestreo invasivo y el no invasivo. El primero implica la captura directa de los individuos para obtener muestras de sangre, tejidos o huesos, lo que garantiza una adecuada cantidad y calidad de DNA para su aislamiento y análisis, aunque puede afectar el bienestar animal (Zemanova, 2020). Se sugiere que una concentración aceptable oscila en 20 – 100 ng/µL de DNA total y su calidad en 1.84 – 2.0 absorbancia para análisis, pero se debe ser cuidadoso en el muestreo y el método de extracción al menos para diferentes mamíferos (Carvajal-Agudelo et al., 2021). También en el tejido se suele obtener una calidad óptima (cercanos a 1.8) con una mayor concentración de DNA total (20 – 40 ng/µL) y menor degradación para cuadrúpedos terrestres (González et al., 2013).

Asimismo, Chacón-Cortés et al. (2011) consideran que las técnicas de extracción de DNA pueden influir en los datos en muestras invasivas (sangre, tejido y hueso), aun así, consideran que los valores aceptables para el análisis de DNA se ubican en un rango de pureza A260/A280 entre 1.7 y 1.9, adecuados para PCR, mientras que la concentración varía según el tipo de muestra, aunque resulta suficiente para aplicaciones moleculares.

El muestreo no invasivo no requiere la manipulación de los ejemplares, ya que se basa en la recolección de muestras oportunas como heces, pelo, saliva y

tejido muerto, lo que representa una alternativa menos intrusiva para los investigadores (Muñoz et al., 2016; Soto-Calderón, 2023). No obstante, este tipo de muestras suelen presentar menor cantidad de DNA (0.4 – 1.5 µg por cada 100 mg de materia fecal) y menor calidad (valores de absorbancia A260/A280 < 1.8) (Marrero et al., 2009). En estudios recientes, sugieren que, aunque dichas muestras tengan valores muy bajos en su calidad pueden mejorarse utilizando técnicas modernas en su extracción y ser más económicas al momento de la colecta (Burgess et al., 2022; Fernandes, 2022). En muestras históricas es importante la preservación de la colección biológica, debido a que se puede realizar estudios génicos en garras, tejidos, pelo y entre otras más (Dunnum et al., 2018; McDonough et al., 2018).

Por lo antes mencionado, es de suma importancia considerar dichos muestreos (invasivo y no invasivo) debido a la rápida degradación de DNA que puede producir perdida de alelos, fragmentación, alelos nulos y baja concentración en la muestra. Que en algunos casos puede ser a causa del traslado, colecta y conservación de la muestra que en general complica los estudios genéticos poblacionales y de conservación en mamíferos (Ackerman, 2014; Zemanova, 2021; Fernandes et al., 2024). En la comparación entre muestras invasivas (sangre y tejido) y no invasivas (heces fecales y pelo) las invasivas resultan más adecuadas. No obstante, los estudios señalan que las muestras invasivas a pesar de sus limitaciones en cuanto a calidad y cantidad de DNA, la información obtenida puede ser confiable para análisis genéticos en mamíferos terrestres (Fernandes et al., 2020; García y Miranda, 2024; Oliveira et al., 2024).

Cabe destacar que las heces fecales constituyen una fuente biológica adecuada para estudios metagenómicos, siempre que se empleen métodos de preservación apropiados que mantengan la estabilidad y calidad del DNA (Guan et al., 2021). Asimismo, diferentes estudios evidencian que las muestras fecales, al menos en cérvidos de libre pastoreo, revelan un microbiota intestinal más diversa y equilibrada, destacando su valor como herramienta no invasiva para evaluar la salud intestinal y los efectos del manejo animal (Wu et al., 2021;

Parsons et al., 2021; Stothart et al., 2021).

No obstante, las muestras fecales y las metodologías metagenómicas también se han aplicado en ganado bovino, mostrando que la dieta de pastoreo influye en la diversidad y función del microbioma, con implicaciones en la eficiencia productiva y en los procesos digestivos (Conteville et al., 2023; Conteville et al., 2024). De manera similar, en ganado caprino se ha evidenciado que el pastoreo libre favorece una microbiota más diversa y funcional. Estos estudios destacan que la dieta basada en forraje modula la composición microbiana del rumen y el intestino, optimizando la digestión de la fibra y promoviendo un equilibrio metabólico. Además, se ha observado que las cabras en condiciones ferales presentan una menor carga de genes de resistencia antimicrobiana, lo que resalta los beneficios del libre pastoreo en la salud intestinal y en la conservación de comunidades microbianas estables (Martínez-Hernández et al., 2022; Li et al., 2023; Li et al., 2014).

El análisis comparativo del microbiota intestinal entre fauna silvestre y ganado bovino y caprino en ecosistemas áridos y semiáridos ha mostrado diferencias relevantes asociadas al tipo de dieta y al manejo. En ganado de pastoreo, las muestras fecales han evidenciado que la dieta forrajera modula la diversidad y función del microbioma, optimizando la digestión de la fibra y manteniendo un equilibrio metabólico, mientras que en rumiantes silvestres se observa un microbiota más diversa y funcionalmente enriquecida, reflejo de la variabilidad dietaria y de la adaptación a entornos naturales (Martínez-Hernández et al., 2021; Glendinning et al., 2021; Molotzu et al., 2024). En este sentido, para el borrego berberisco (*Ammotragus lervia*), las muestras fecales, aunque con cierto grado de degradación, han resultado viables para estudios de variabilidad genética en poblaciones silvestres (Pizzigalli et al., 2023; Beja-Pereira et al., 2004).

Con base en lo anterior, la determinación del microbioma fecal de *Ammotragus lervia* y ganado doméstico en el APFF Cañón de Santa Elena, Chihuahua, México se plantea como una estrategia que permitirá evaluar la diversidad microbiana en un contexto de coexistencia entre fauna silvestre y especies domésticas en pastoreo. Este enfoque, enmarcado en las limitaciones

técnicas del uso de muestras invasivas y no invasivas en mamíferos terrestres, resalta el valor de las muestras fecales como una alternativa no invasiva que, pese a sus limitaciones de degradación, permite obtener información confiable sobre la salud intestinal y la dinámica ecológica, contribuyendo al mismo tiempo a la conservación y al manejo sustentable de este ecosistema árido.

1.2 OBJETIVO GENERAL

Analizar las limitaciones técnicas del uso de muestras invasivas y no invasivas en mamíferos terrestres, y determinar el perfil del microbioma fecal del borrego berberisco (*Ammotragus lervia*) frente al ganado bovino y caprino en el APFF Cañón de Santa Elena, Chihuahua, México.

1.3 OBJETIVOS ESPECIFICOS

- Realizar un análisis cienciométrico para reconocer a los principales autores, tendencias y líneas de investigación que utilizan muestras invasivas y no invasivas en estudios de genética en mamíferos terrestres.

- Mediante una investigación documental se identificarán y discutirán la calidad de DNA en muestras invasivas y no invasivas en mamíferos terrestres para determinar su variabilidad genética.

- Determinar, mediante muestreo no invasivo, la composición e interacción del microbioma fecal del borrego berberisco (*Ammotragus lervia*) frente al ganado bovino (*Bos taurus*) y caprino (*Capra aegagrus hircus*) que cohabitan en el APFF Cañón de Santa Elena, Chihuahua, México.

1.4 HIPOTESIS GENERAL

Ha: Existen diferencias en la calidad de DNA obtenido de muestras invasivas y no invasivas en mamíferos terrestres. Así mismo existen diferencias en la composición del microbioma fecal del borrego berberisco (*Ammotragus lervia*) y frente al ganado bovino (*Bos taurus*) y caprino (*Capra aegagrus hircus*) que cohabitan en el APFF Cañón de Santa Elena, Chihuahua, México.

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CHAPTER 2. THE EVOLUTION AND SCOPE OF INVASIVE AND NON-INVASIVE SAMPLING IN TERRESTRIAL MAMMAL POPULATION GENETICS: IMPLICATIONS FOR THE COMPARABILITY OF THE He, Ho and Fis: A SCIENTOMETRIC REVIEW.

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Review

The Evolution and Scope of Invasive and Non-Invasive Sampling in Terrestrial Mammal Population Genetics: Implications for the Comparability of He, Ho and Fis: A Scientometric Review

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Abstract

This scientometric review examines the evolution and scope of invasive (blood and tis- sue) and non-invasive (faeces, hair, and saliva) sampling in terrestrial mammal popula- tion genetics, with particular emphasis on the comparability of observed heterozygosity (Ho), expected heterozygosity (He), and the inbreeding coefficient (Fis) between stud-ies published from 1985 to 2026. Searches in Web of Science and Scopus, filtered under PRISMA/PRISMA- S criteria, yielded a compendium of articles analysed with Bibliometrix and VOSviewer 1.6.20 to quantify temporal production, keyword evolution, collaborative networks, and publication outlets. Searches in Web of Science and Scopus, filtered under PRISMA/PRISMA-S criteria, yielded a broad corpus of 145 articles for general sciento-metric analyses, of which 85 met the eligibility criteria for the focused analysis of Ho, He, and Fis. The field shows steady growth (annual rate ≈ 6.1%), substantial authorship and international collaboration, and increasing thematic diversity. Adoption of non-invasive sampling has accelerated, broadening spatial and taxonomic coverage, but also increasing exposure to DNA degradation and genotyping error when laboratory quality control is insufficient. Across the literature, reporting of quality control practices (e.g., extraction blanks, negative PCR controls, multi-tube replication, and error-rate estimation) has im-proved over time but remains inconsistent. Comparisons indicate that differences in Ho, He, and Fis between invasive and non-invasive sampling are generally modest once marker system and species are taken into account. These findings indicate that quality control and transparency in reporting, rather than invasiveness per se, are the main factors determining comparability among studies. The scientometric patterns also reveal a methodological transition from microsatellites to SNP-based and reduced representation approaches, with implications for synthesis across marker types. Overall, this review identifies geographic and taxonomic biases in research effort and highlights the need for standardised reporting of DNA quality indicators, inclusion thresholds, and validation protocols to strengthen genetic monitoring in mammalian conservation.

Keywords: genetic tracking; quality control protocols; bibliometric mapping; co-authorship networks; SNP-based genomics

1. Introduction

Habitat loss, fragmentation and other anthropogenic pressures continue to erode the genetic diversity of wild mammal populations, with consequences for demographic viability, adaptive potential and extinction risk [1,2]. In conservation and population genetics, a basic set of metric-observed statistics—heterozygosity (Ho), expected heterozygosity (He) and inbreeding coefficient (Fis)—provides comparable indicators of genetic status and deviations from Hardy–Weinberg equilibrium at loci [3,4]. These statistics inform management by revealing excess homozygosity (Fis > 0) or heterozygosity (Fis < 0) and by flagging populations at risk of genetic erosion. It is critical to note that the reliability and comparability of Ho, He and Fis estimates depend on how the DNA is obtained. Invasive samples (e.g., blood and tissue biopsies) often produce high-integrity DNA, which minimises genotyping errors, but they require animal capture and pose ethical and logistical constraints [5]. Non-invasive samples (faeces, hair, and saliva) extend spatial and taxonomic coverage, especially for elusive or threatened species, but often contain low-quality or degraded DNA, raising the risks of allele loss and false alleles, unless strict laboratory and bioinformatics security measures are in place [6,7]. As genetic monitoring gains importance in conservation policy and assessment (e.g., integration of genetic indicators into threat assessments), understanding how the mode of sampling shapes the literature and the metrics that are published is of immediate practical relevance [8]. Despite the plethora of case studies covering marker systems from microsatellites to SNP panels, no field-level synthesis has been conducted that reflects how the choice between invasive and non-invasive sampling has influenced scientific productivity, collaborative networks, keyword trajectories and, most importantly, the comparability of He, Ho and Fis in terrestrial mammal studies. Scientometric tools such as Bibliometrix and VOSviewer allow for quantitative scientific mapping of temporal production, co-authorship structure and thematic co-occurrence to address precisely these questions [9,10]. In this paper, we conduct a scientometric analysis (1985–2025) of terrestrial mammal studies reporting on Ho, He and Fis, paying particular attention to the mode of sampling (invasive vs. non-invasive). Specifically, we (1) characterise temporal trends in publication volume and emerging themes; (2) identify the countries, institutions and journals driving production and describe the architecture of their collaborative networks; and (3) quantify the expansion of non-invasive sampling and assess its implications for DNA quality control, genotyping error management and inter-study comparability of key genetic metrics. By integrating scientific mapping with targeted assessment of sampling practices, our synthesis provides an evidence-based agenda for standardising DNA quality indicators, reporting thresholds and validation protocols, thereby improving the robustness and interoperability of genetic monitoring in mammalian conservation. In this paper, we conduct a scientometric review of terrestrial mammal studies published between 1985 and 2025 that used invasive or non-invasive samples in conservation-oriented population genetic research. The primary objective of the study is descriptive: to map temporal trends in publication output, thematic development, geographic participation, collaboration net-works, and journal patterns in this field. As a complementary objective, we examine how sampling mode, marker system, and quality control practices have been reported in the subset of studies that included Ho, He, or Fis, in

order to assess the methodological con-ditions under which these indices are interpreted and compared in the literature. Thus,the manuscript is intended principally as a scientometric mapping exercise with a focused methodological synthesis.

2. Materials and Methods

We followed the PRISMA 2020 and PRISMA-S guidelines [11] for literature searching and reporting (Figure 1). A detailed protocol (search strings, selection form, codebook and R scripts) was prepared a priori. Explanatory note: The difference between the 85 studies retrieved using PRISMA and the 145 articles reported in the Results Section is that only the former met the eligibility criteria for the analysis of Ho, He and Fis. The latter set was used to describe general publication and scientometric patterns. Consequently, the total number of articles is not listed in the References Section; only articles supporting the main focus of this review are included.

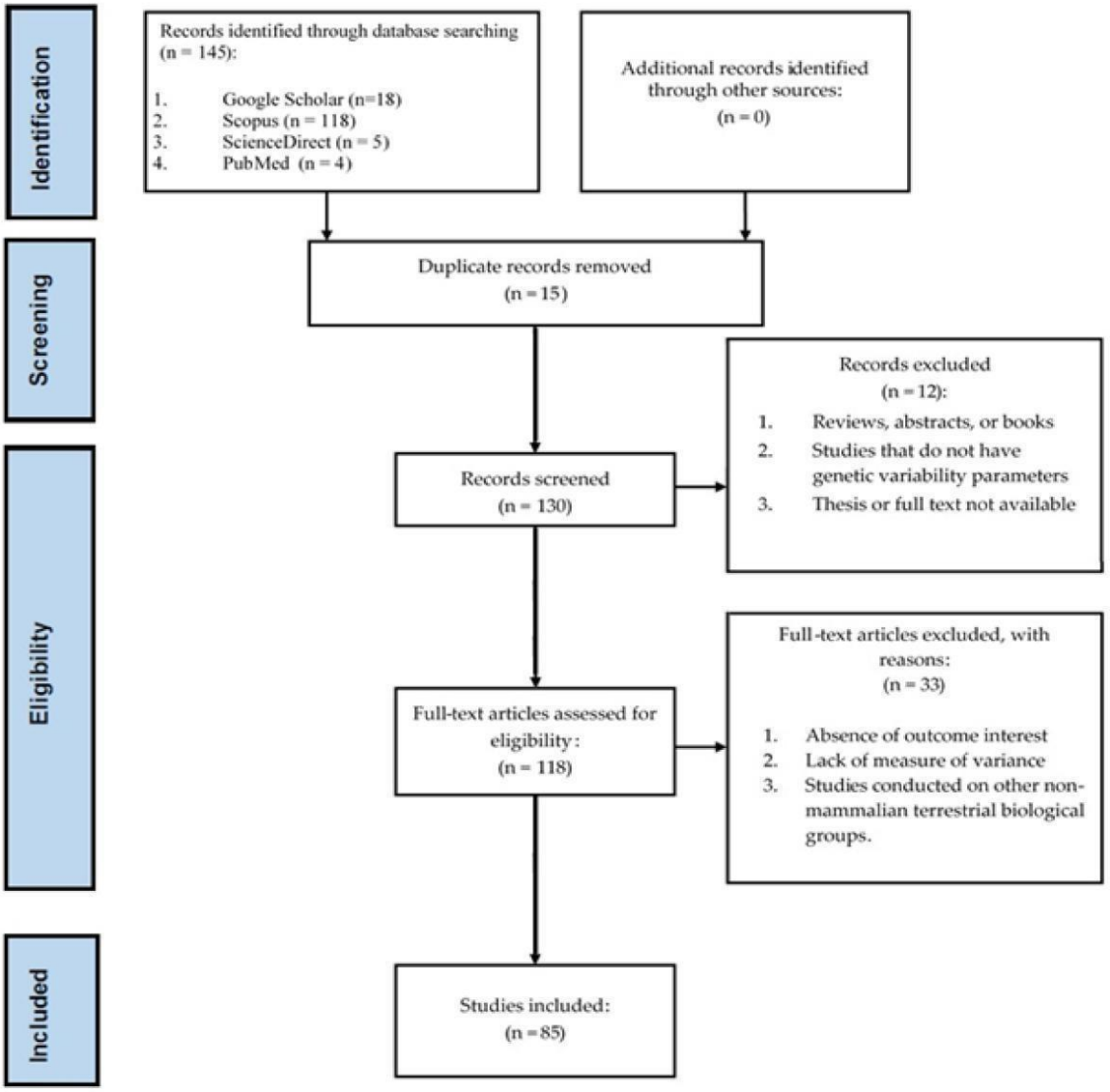


Figure 1. PRISMA flow chart showing the literature search strategy and study selection in this scientometric review.

This review was organised around two nested datasets. The first was a scientometric corpus of 145 articles, used exclusively for analyses of publication trends, keyword evolu-

tion, collaboration networks, countries, institutions, and journal patterns. The second was a focused analytical subset of 85 studies, comprising those articles from the broader corpus that explicitly reported at least one of the population genetic metrics—Ho, He, or Fis—and met the eligibility criteria for the targeted methodological synthesis. Unless otherwise stated, bibliometric and network-based analyses were conducted using the 145-article corpus, whereas analyses and descriptive syntheses related specifically to Ho, He, and Fis, sampling- mode classification, and quality control reporting were based on the 85-study subset or on the corresponding classified records within the broader corpus, as indicated in each section.

2.1. Eligibility Criteria

Population and scope: We focused on studies on terrestrial mammals (class: Mammalia) in free-ranging or reintroduction contexts that reported any of the following popu- lation genetic metrics: observed heterozygosity (Ho), expected heterozygosity (He) and inbreeding coefficient (Fis). Study designs: Primary research reporting empirical genetic data from wild populations (including museum/archival specimens) using any marker system (microsatellite/SSR, SNP, mitochondrial DNA, nuclear sequences, reduced rep- resentation sequencing, and WGS) were eligible. Mode of sampling: Items had to allow for classification of the sample type as invasive (blood, tissue/biopsy, ear notch, mus-cle, liver, and skin, including remote dart biopsy) or non-invasive (faeces/excreta, hair, saliva/chewed material, urine, and shed skin). Exclusions: Marine mammals; exclusively captive or laboratory colonies without wild origin; purely methodological articles lacking field data; reviews, editorials, theses and conference abstracts; studies without Ho, He or Fis; and non-mammalian focal taxa were excluded.

2.2. Sources of Information and Dates of Coverage

The Web of Science Core Collection (WoSCC) and Scopus were searched from 1 January 1985 to 1 March 2026. Only scientific writings in English were used.

2.3. Search Strategy (Exact and Reproducible)

We used controlled Boolean expressions targeting population genetic metrics, mammalian and typical sampling keywords. Field labels reflect the syntax of each database.

Marine filter: Marine mammals were excluded during selection using habitat fields and title/summary clues (e.g., “pinniped”, “cetacean”, and “marine”).

De-duplication: Before selection, combined exports were merged by DOI (case-insensitive). Records without DOIs were merged using exact title matching after Unicode normalisation and then by approximate title matching (Levenshtein ratio ≥ 0.92 with the same main author and year).

2.4. Selection Workflow

Selection was carried out in two stages using a pre-screened form (title/abstract → full text). Two independent reviewers screened all records; disagreements were resolved by consensus or with the help of a third reviewer. Inter-rater reliability for inclusion decisions at the title/abstract stage was quantified with Cohen’s κ coefficient (95% CI).

2.5. Data Extraction

For each included article, two reviewers extracted independently:

Bibliographic fields: DOI, title, year, journal, country of corresponding author, all author affiliations (subsequently harmonised by country), funding text (when available), and open access status.

Study descriptors: focal species (scientific name), continent/region, biome, sample size (per population), number of populations, and year(s) of sampling.

Genetic methods: marker system; genotyping platform; number of loci (for SSR) or SNP; filtering thresholds (e.g., MAF and missing data); HWE testing and multiple testing correction.

Sampling mode (primary variable): invasive vs. non-invasive vs. mixed vs. unclear (see operational definitions below).

Quality control (QC) indicators (binary/quantitative): replication rate, allele loss estimates, false allele rate, use of multiple tubes for non-invasive DNA, negative controls, extraction targets, authentication steps for old/archived DNA, and reported genotyping error model/use of PEDANT/STaRT.

Population genetic metrics: Ho, He, and Fis (as reported); number of loci used in their calculation; and values per population when available.

Discrepancies were resolved by discussion. If a study reported on multiple populations, the study population was treated as the unit of analysis for metric distributions; bibliometric analyses were kept at article level.

Operational definitions: sampling mode

Invasive: any procedure that pierces the skin or removes tissue (including remote dart biopsy); includes blood draws; or removes organs or tissues from euthanised animals.

Non-invasive: DNA obtained without capturing/handling animals (faeces/excreta, hair traps, saliva/chewed sticks, urine, and shed skin).

Mixed: both modes were used with an analysable breakdown.

Unclear: insufficient detail after review of full text and supplements.

Ambiguities were resolved using method sections and Supplementary Materials; where still unclear, correspondence sections were consulted for clarification.

2.6. Data Curation and Harmonisation

Species names were standardised according to the Mammal Species of the World/ITIS spelling. Author and institution names were disambiguated using ORCID, ROR, GRID and string distance clustering with manual verification for high-degree nodes. Country names were harmonised with ISO-3166-1 alpha-3 codes. Open access status was assigned using DOI searches with Unpaywall exports (where available in metadata dumps) and journal policies.

2.7. Bibliometric and Scientific Mapping Analyses

All analyses were performed in R 4.3.x with Bibliometrix (≥4.3) and bibliometrixData, complemented by VOSviewer 1.6.20 for network visualisation.

Performance analysis: annual scientific output; top journals, countries, institutions and authors; and collaboration indices (authors/article and institutions/article).

Co-authorship and cross-country collaboration networks: fractional counting; edges

were preserved if ≥2 co-publications; labels were limited to the top N = 50 nodes per degree, unless otherwise stated.

Keyword analysis: authors' keywords plus Keywords-Plus (WoS)/index terms (Scopus) after lemmatisation and removal of empty words; a thesaurus was applied to merge synonyms (e.g., "microsatellite(s)" ↔ "SSR", "STR"). Minimum occurrence threshold = 5 (sensitivity: 3– 10).

Thematic evolution and trending themes: 5-year moving windows; K = 50 main keywords per window; Sankey plots for thematic evolution.

Thematic map: Callon centrality versus density with predetermined clustering (Bibliometrix), using normalised weights per field.

2.8. Software, Versions and Computational Environment

Core packages: Bibliometrix ≥ 4.3, tidyverse ≥ 2.0, stringdist ≥ 0.9, countrycode ≥ 1.6, mgcv ≥ 1.9, lme4 ≥ 1.1, nlme ≥ 3.1, and irr ≥ 0.84. Visualisations were performed in VOSviewer 1.6.20 and ggplot2. Random seeds were set to 12,345 for any stochastic routines (e.g., cluster initialisation). Operating system: Windows 10.

2.9. Ethical Statement

This study analysed the published literature only; no animals or human subjects were used.

3. Results

The publications analysed covered the period from 1985 to 2024 and comprised 145 scientific articles. The annual growth rate was 6.08%, and the mean number of citations per article was 51.31, indicating sustained scientific interest in the topic. A total of 400 author keywords were identified, reflecting substantial thematic diversity in this field.

3.1. Scientific Production over Time

Despite an annual growth rate of 6.08% in publications, no scientific production was recorded between 1985 and 2002 (Figure 2). This is probably because the basis for estimating inbreeding coefficients was still being investigated during this period. Between 2003 and 2011, there was an increase of 32 scientific articles (27% of the total), with an average of 3–4 articles per year.

However, between 2012 and 2024, around 54 studies were published, equivalent to 45% of the publications, with an average of 5–8 articles per year, which places this topic as one of current interest.

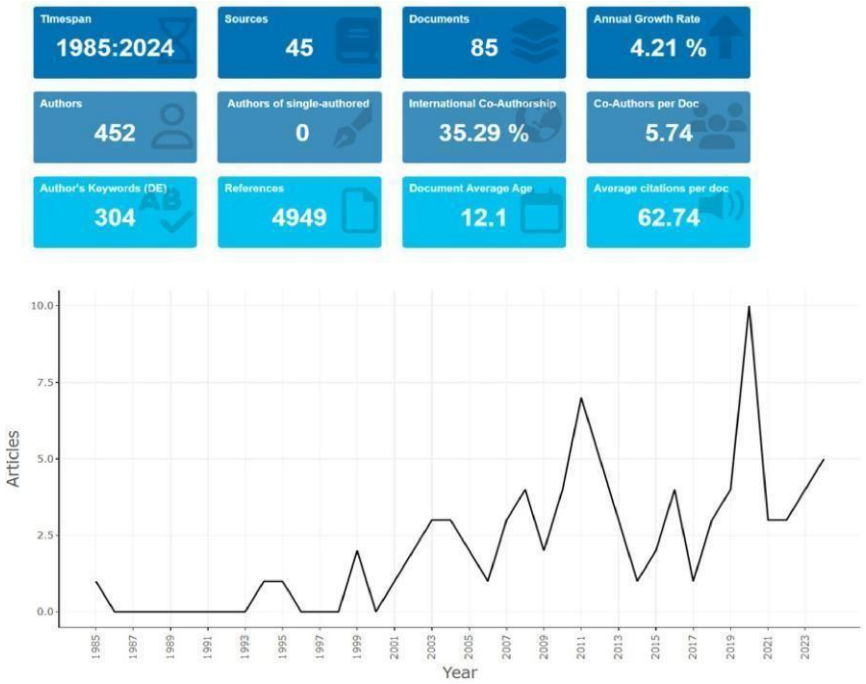


Figure 2. Scientific production over the years of articles related to the estimation of genetic variability through inbreeding coefficients for mammals.

3.2. Distribution and Temporal Trends of Invasive and Non-Invasive Sampling

Of the 145 articles included in the scientometric corpus, 34 used invasive samples, 21 used non-invasive samples, 64 used mixed sampling designs, and 26 did not provide sufficient methodological detail for unambiguous classification. Within the focused sub- set of 85 studies that reported Ho, He, or Fis, 23 were based on invasive samples, 12 on non-invasive samples, and 33 on mixed designs, and 17 were classified as unclear. Over- all, non-invasive sampling became more frequent in the later years of the study period, particularly after [2010–2024], whereas invasive sampling predominated in earlier studies. This descriptive pattern is consistent with the increasing adoption of minimally disruptive genetic monitoring approaches in terrestrial mammal research.

3.3. Keyword Concurrence

In this analysis, 76 different keywords were obtained, with a network of 46 main words with a minimum occurrence of 10 times, from the total dataset of the WoSCC database. The first group (green) consists of 15 keywords with terms such as loci, microsatellite and marker. The second group (yellow) consists of 10 words mainly related to the words population and genetic variability. The third group (blue) is made up of nine keywords in which the main concept is genetic variability. In the case of groups 4, 5 and 6 (purple, strong blue and aqua green), there are five keywords in groups 4 and 5 and two keywords in group 6, the most referential concepts for these groups being sample, polymorphism and allelic richness. According to the history of the population and the use of each of the words in 2008, words such as genetic variability, genetic variation, distance, hybridisation and low level were used. Subsequently, in the period 2010–2012, the most used words were population, microsatellite, loci, differentiation and variation. According to the mapping in the period 2014–2016, terms such as sample, marker, subpopulation, reintroduction, allelic richness and habitat fragmentation were used (Figure 3).

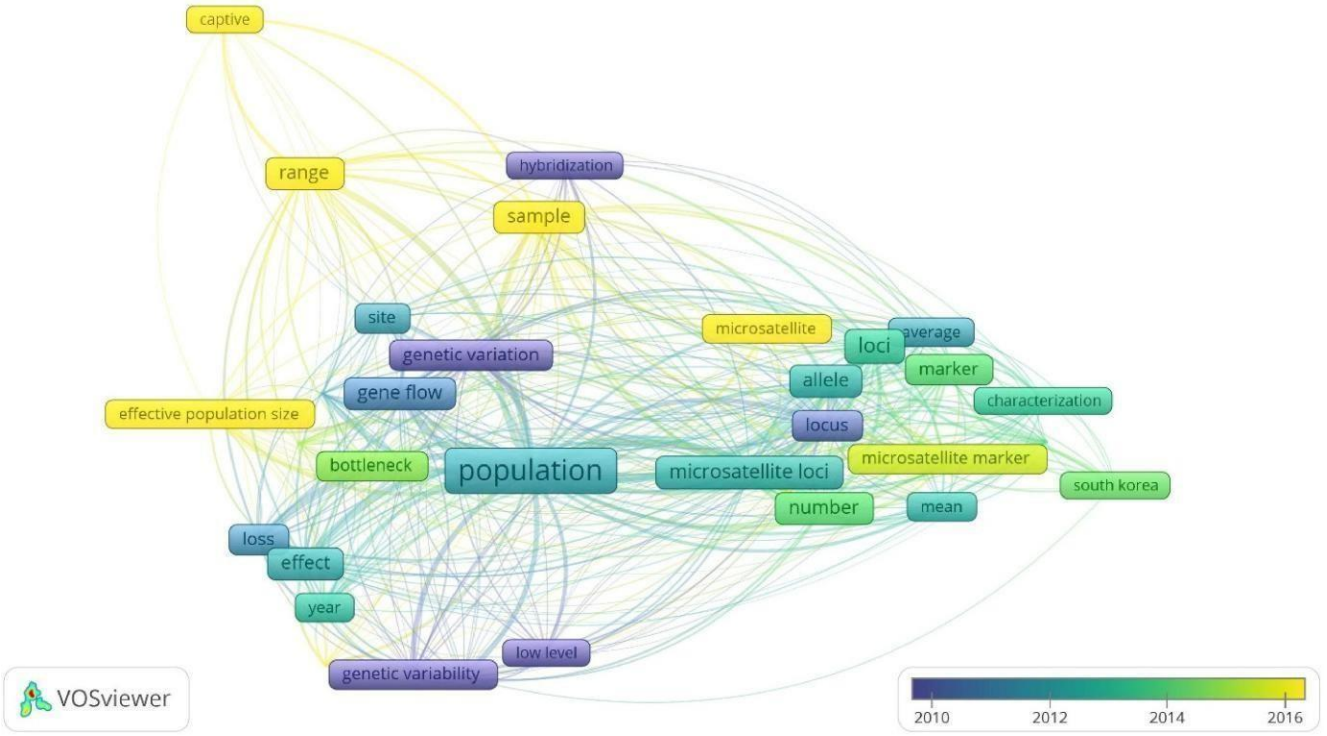


Figure 3. Keyword matching in published articles on estimating genetic variability through inbreed- ing coefficients for mammals.

3.4. Analysis of Author Productivity and Collaborative Networks

The Lotka plot shows that most authors wrote between one (93.1%) and two articles (6.1%) (Figure 4). The author with the most published papers was Paetkau D., with four papers between 1995 and 2020. He was followed by the author An, J., with three contributions in the annual period 2011–2018; Shen-Guo, F., in 2008–2010; Galleti, J.R., in the period 2011– 2022; and Ettore, R., with the same production, but in a longer period, 1999– 2021 (Figure 5).

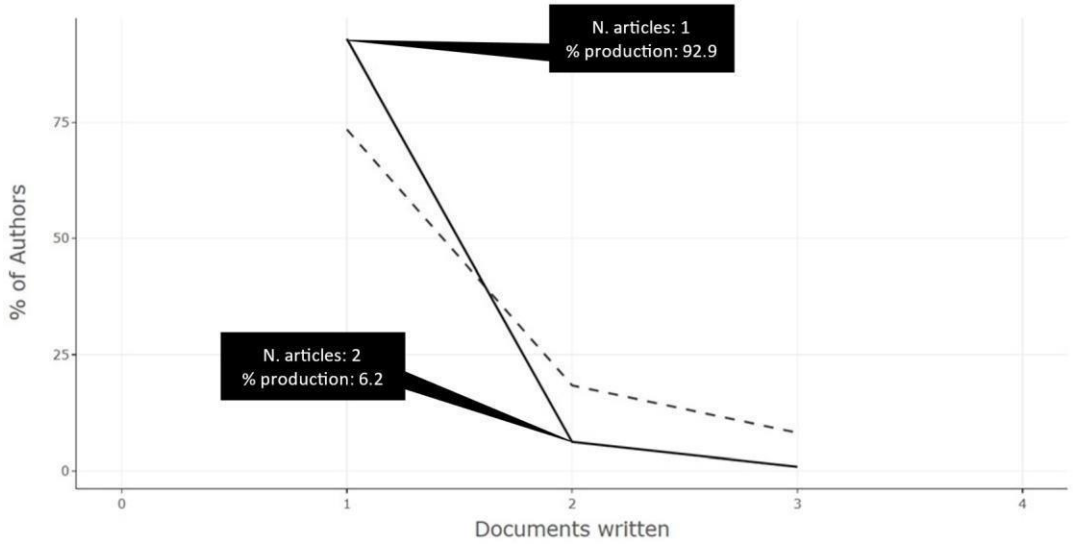


Figure 4. Scientific production of researchers related to the topic of genetic variability through inbreeding coefficients for mammals according to Lotka's law.

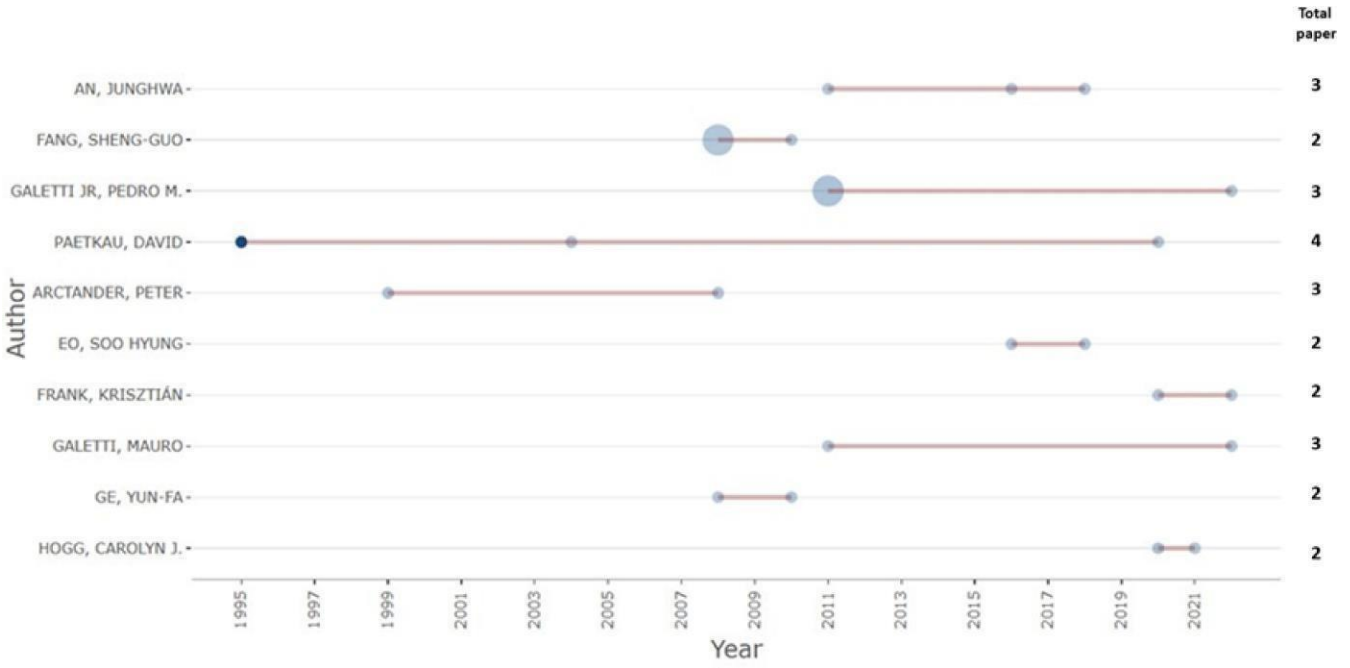
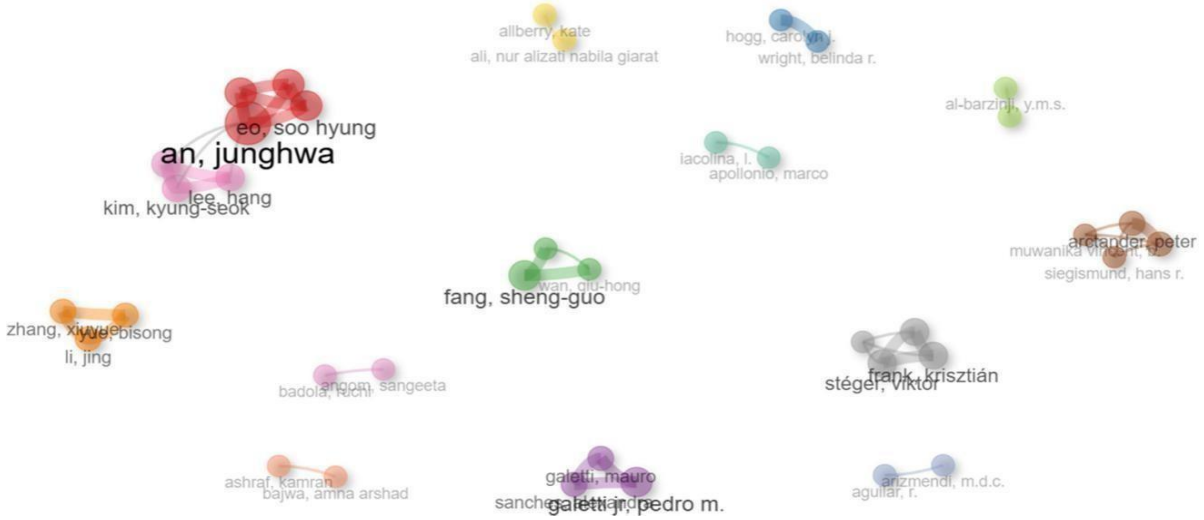


Figure 5. Scientific production related to genetic variability through inbreeding coefficients for mammals, according to the 13 most productive researchers. The solid line represents the trajectory of the researcher. The size of the circle reflects the annual production, and the intensity of the colour indicates the number of citations received that year.

The authors with the longest track record were Paetkau D., Ettore, R. and Arctander, P., who maintain a low but extensive output due to the number of years of research, at least



in this type of study. The above-mentioned researchers did not collaborate with each other, as they each maintained their own working groups (Figure 6).

Figure 6. Collaborative networks investigating genetic variability through inbreeding coefficients in mammals.

3.5. Global Scientific Collaboration

The countries publishing the most studies on the estimation of inbreeding coefficients in invasive and non-invasive mammalian samples were the United States, China, Italy,

Canada, the United Kingdom, and France. Figure 7 illustrates the international collaboration network derived from co-authorship affiliations among countries. In this map, node size reflects the relative scientific output of each country, while the connecting lines represent collaborative links between countries based on co-authored publications. The brown lines indicate the existence and relative intensity of these international collaborations; thicker or more prominent links denote stronger collaboration based on a greater number of shared publications. Thus, the figure should be interpreted as a visual representation of the structure and strength of cross-country scientific cooperation in this research field. (Figure 7).

3.6. Scientific Production by Indexed Journals

The analysis of scientific production over time on the estimation of inbreeding coefficients in invasive and non-invasive samples showed that at least five journals have made important contributions to this research topic (Figure 8).

Between 1994 and 2023, the most efficient journal on these topics was *Molecular Ecology*, with more than 15 publications in total. This growth was remarkable in the period 2003–2009, from 4 to 13 scientific articles. Other journals on similar topics have stood out for their scientific articles, such as *Conservation Genetics*, which experienced significant growth in 2011 and, to date, leads the trend in the publication of scientific articles on this topic. However, the *Journal of Mammalogy* maintained a population trend of five articles per year between 2012 and 2023. On the other hand, in 2014, the *Journal of Wildlife Management* experienced an increase in its scientific production, as did *Molecular Biology Reports*, but in more recent years (2020–2023).

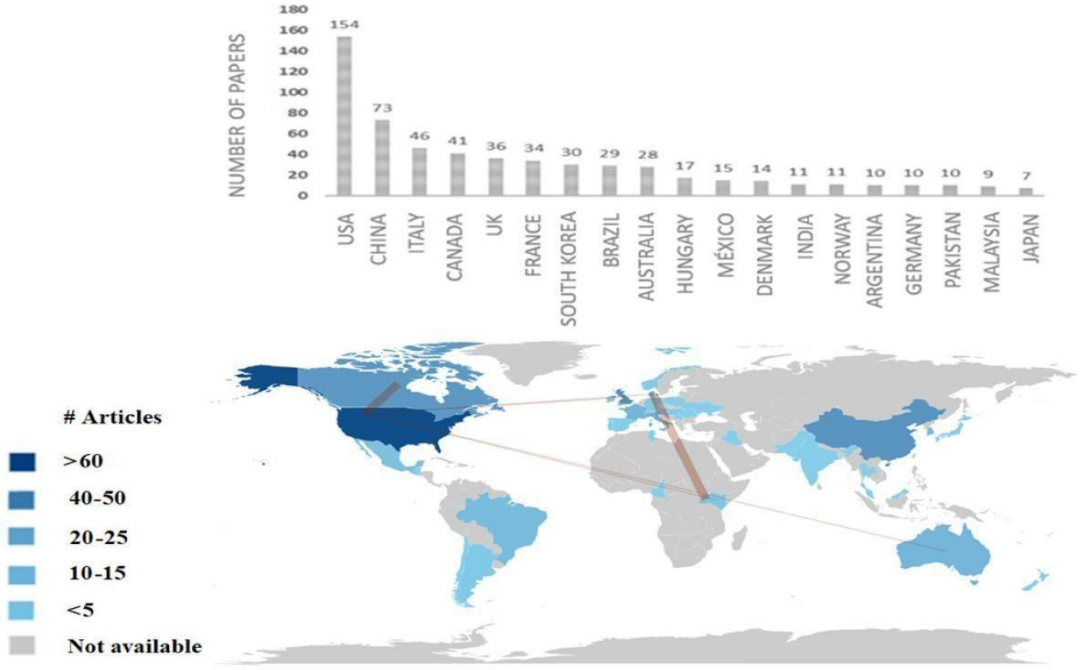


Figure 7. International scientific production and collaboration network in studies addressing genetic variability and inbreeding coefficients in mammals. Node size represents the relative scientific output of each country. Connecting lines represent collaborative links between countries based on co-authored publications; the brown lines indicate international collaborations, and their prominence reflects the relative strength or frequency of these collaborations. The colour intensity of the map reflects the level of collaboration among countries.

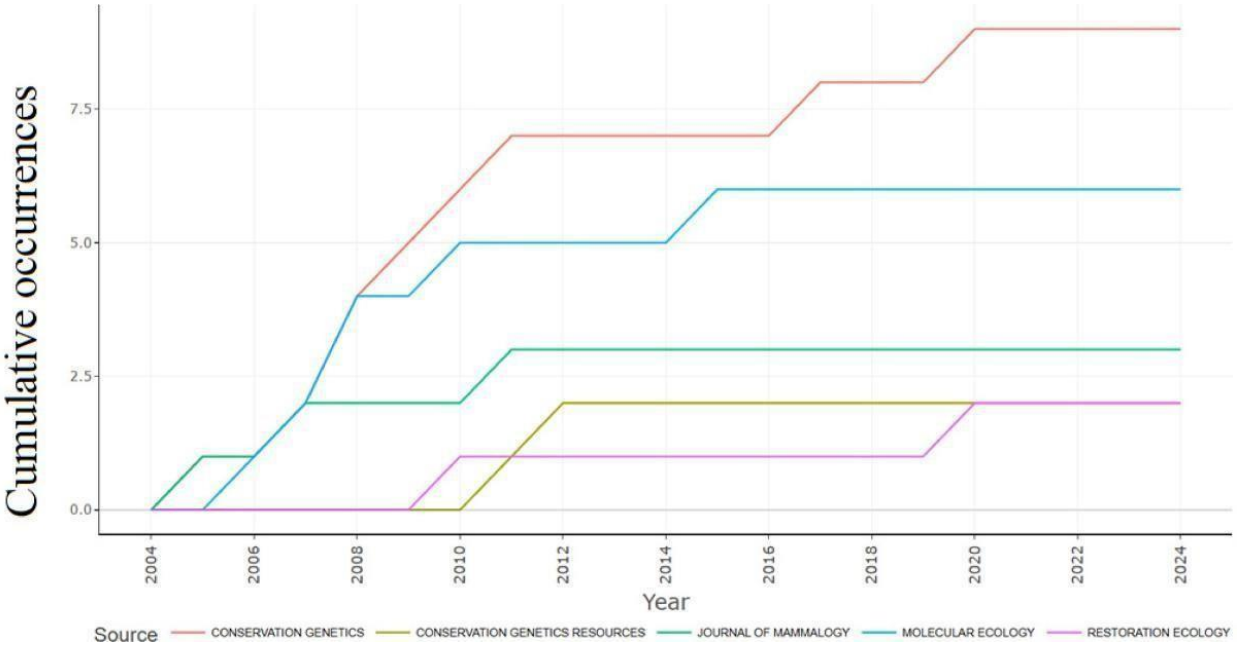


Figure 8. Scientific production related to the topic of genetic variability through inbreeding coefficients for mammals by the main specialised journals.

4. Discussion

Taken together, these findings should be interpreted primarily as evidence of how the field has developed bibliometrically and how methodological practices have been reported, rather than as a formal quantitative demonstration of causal differences in Ho, He, or Fis between sampling modes.

4.1. Expansion of Non-Invasive Sampling and Its Consequences for Coverage

Our analysis shows a pronounced and sustained increase in the use of non-invasive sampling (e.g., faeces, hair and saliva) in terrestrial mammal studies, especially in the last 10– 15 years. Conceptually, this shift reflects the long-recognised benefits of minimising animal handling while expanding spatial and taxonomic coverage, advantages highlighted in seminal reviews on non-invasive genetics [12,13]. Ethically, the trend is in line with contemporary applications of the 3Rs in wildlife research, which encourage refinement or replacement of invasive practices where equivalent data quality can be achieved [8]. In practice, greater acceptance of non-invasive approaches is likely to have enabled work on elusive or threatened taxa and in jurisdictions with stricter permits [14]. The implication is a more inclusive evidence base for conservation genetics; however, these benefits depend on rigorous quality control (QC) to manage degraded DNA and contamination risks inherent in non-invasive substrates [14,15].

4.2. Quality Control Reporting: Improvements, Remaining Gaps and Impact on Genetic Metrics

We observed improvement in quality control reporting over time (e.g., extraction blanks, negative PCR controls, and replication of low-quality extracts), but documentation remained inconsistent across studies [8]. This is important because allele loss and false alleles arise from suboptimal PCR and low-quality or degraded DNA, inflating homozygosity and biassing Fis upwards, which could mimic inbreeding or Wahlund effects if not

accounted for [16]. Suboptimal PCR conditions can produce stutter patterns, spurious peaks, overloaded amplicons and signal saturation, all of which can mask and distort the genuine allelic signal during fragment analysis, whereas degraded DNA can produce allelic dropout, false alleles and unbalanced heterozygotes. Empirical evaluations show that the adoption of multi-tube replication, consensus genotyping, explicit error rate estimation and inspection of raw reads significantly improve reliability in non-invasive datasets [14]. Therefore, theoretical and practical orientations converge: journals and reviewers should require transparent quality checklists during the allele analysis workflow (replication criteria, genotyping error quantification, read-level verification, and data curation steps), as the interpretability of Ho, He and Fis depends on both laboratory practice and field sampling mode [13].

In this regard, it is essential to establish a clearer distinction between the sampling method itself and the quality control framework applied to the resulting DNA [17]. In the field of non-invasive genetics, degraded DNA or DNA with low template content is particularly vulnerable to allele loss and false alleles. These errors can directly distort population genetic estimates if not addressed through the implementation of structured analytical and laboratory safeguards. Allele loss is a process that has the effect of converting true heterozygotes into apparent homozygotes, which in turn tends to reduce observed heterozygosity (Ho) while leaving expected heterozygosity (He) comparatively less affected. This process can result in an inflation of F_i, which in turn can generate spurious signals of inbreeding or Hardy– Weinberg imbalance [16].

From this standpoint, the organisation of robust quality control in non-invasive genetics can be conceptualised around four fundamental components. Firstly, the replication strategy is to employ repeated amplification of low-quality extracts and, where appropriate, multi-tube approaches to reduce stochastic genotyping errors. Secondly, regarding estima- tion of error rate, studies must explicitly quantify allele loss and false allele rates so that the reliability of genotypes can be assessed transparently. Thirdly, consensus genotyping is to be advocated for, whereby final multilocus genotypes should be based on predefined consensus rules rather than on single amplifications, especially when working with de- graded DNA. Fourthly, it is imperative that reports specify the thresholds employed for locus and sample inclusion, missing-data filtering, and genotype acceptance. In addition, any identity- based criteria, such as PID siblings, must be specified when individual dis- crimination is relevant. The classical methodological literature has long emphasised the need for replication and formal error assessment in low-quality DNA. More structured non-invasive workflows have demonstrated that reliable genetic inference can be achieved when these safeguards are rigorously applied. For instance, Zarzoso-Lacoste et al. [17] provide a clear implementation of a structured QC framework in non-invasive mammalian genetics, incorporating replication, error estimation, and explicit validation criteria. In this context, the central interpretive issue is not simply invasive versus non-invasive sampling, but the degree of methodological stringency used to authenticate degraded DNA datasets and to document residual uncertainty.

4.3. Comparability of Ho, He and Fis Between Sampling Modes After Accounting for Methods

When we accounted for the marker system and species as random effects, differences in Ho, He and Fis distributions between invasive and non-invasive surveys were modest. This pattern is consistent with theory: the mode of sampling itself should not alter

pop- ulation genetic parameters if genotyping error is minimised and filtering and reporting are standardised [15–18]. Apparent deficits in heterozygosity or excess homozygosity are more parsimoniously explained by technical artefacts, null alleles or population substruc- tures, rather than by the invasiveness of sampling per se [19]. The practical implication is twofold. First, syntheses across studies should be stratified by marker type and level of quality control before drawing biological conclusions. This is essential because SSR and SNP differ fundamentally in genomic density, allele spectra and mutation dynamics and therefore, metrics such as Ho, He and Fis are not directly comparable. Second, authors should clearly separate the biological signal (e.g., true inbreeding) from the methodological signal (e.g., thresholds of missing data, data processing and null allele detection could vary among studies, further influencing estimates of genetic diversity and inbreeding), following recommended diagnostics and reporting conventions [18,19].

4.4. Transitioning the Marker System: From SSR to SNP and Reduced Representation Sequencing

The transition from the microsatellite (SSR) field to SNP panels through genome reduced representation and whole genome sequencing follows in the wake of broader advances in ecological and conservation genomics [16–19]. This transition has a direct influ-ence on the scientometric patterns of our dataset: as SNP-based studies proliferate, driven by the decreasing cost of the genotyping platforms, reported diversity and structure metrics increasingly reflect high-density genotyping with different ascertainment properties that differ from those of SSRs [8]. While SNPs provide superior genome-wide coverage and reproducibility, comparisons of Ho/He between marker systems should be approached with caution, as absolute values and variance may differ due to mutation patterns, allelic spectra and underlying mutation models [18]. Future syntheses would benefit from marker-specific summaries or databases, in which studies based on microsatellites and SNPs are organised and interpreted separately, with explicit cross-referencing only when compar-isons across marker systems are justified. This approach would improve transparency
and reduce overinterpretation when integrating datasets generated with different genetic technologies [16–20].

4.5. Geographic and Taxonomic Bias in the Research Effort

We detected persistent regional and taxonomic imbalances, for example, the concentra- tion of results in higher-income countries and in a subset of charismatic or well-resourced mammal clades. These biases reflect broader barriers to global biodiversity research, in- cluding disparities in funding, computational and bioinformatic infrastructure, language and security [21]. Methodologically, non-invasive sampling can partly compensate for logistical and permitting barriers in under-represented regions, but equitable capacity building, incentives for data sharing and multilingual dissemination are also required to diversify the evidence base [13–22]. Policymakers and funders can leverage this knowledge to prioritise support for regions and taxa with the largest knowledge gaps, especially where genetic monitoring could have the greatest impact on conservation outcomes.

4.6. Collaborative Networks, Openness and Consolidation in the Field

Co-authorship networks in our data became denser and more modular over time,

indicating a consolidation of specialised groups and inter-institutional collaborations. This is consistent with patterns identified across scientific mapping workflows and reflects the maturation of conservation genetics towards a more collaborative and methodologically standardised field [9,10]. We also observed a growth in open access publications. This is associated with increased visibility and reusability of curated genomic resources, features that are especially valuable for comparative and follow-up studies [22]. The implication is practical: open methods and shared data and codes accelerate harmonisation of quality control standards and facilitate reproducible syntheses across taxa and regions [10–23].

4.7. Implications for Genetic Monitoring and Conservation Decision-Making

Taken together, our findings support a pragmatic way forward: non-invasive sampling can provide reliable population genetic indicators when combined with rigorous quality control and clear reporting. This allows for more frequent and ethically refined monitoring of at-risk populations [24]. For conservation practice, this means that agencies can adopt standardised quality control checklists and require reporting of error rates, replication criteria and screening thresholds, along with Ho, He and Fis, which is essential for ensuring comparability across studies that rely on different marker systems. On the research side, the community should agree on minimum reporting standards independent of sampling mode (e.g., multi-tube replication for low-quality DNA; missing locus diagnosis and HWE), thereby improving interoperability between studies and the evidentiary value of genetic metrics in management plans [14].

4.8. Limitations and Future Directions

As a scientometric synthesis, our inferences are limited by database coverage, indexing practices and heterogeneity of reporting across journals. Although we applied rigorous de-duplication and disambiguation processes, some affiliation and country assignments remain imperfect. This is an endemic limitation of bibliographic metadata [10]. Future work should integrate full-text mining to capture QC details not present in abstracts, assess how study design modulates the effects of sampling mode on genetic metrics, and jointly develop domain-wide reporting templates with journals and societies to standardise QC methods and disclosure across marker and taxon systems [9,10].

4.9. Recommendations for Future Studies and Reporting Practice

Based on the patterns identified in this scientometric review, we propose several recommendations to improve the comparability, transparency, and utility of terrestrial mammal population genetic studies using invasive and non-invasive samples.

First, studies should report sampling mode explicitly and operationally, including the biological material collected, whether samples were obtained with or without capture or handling, and whether mixed sampling designs were used. Clear classification of sample origin is essential for comparing methodological approaches across studies.

Second, minimum quality control information should be reported routinely, particularly for non-invasive and low-quality DNA sources. At a minimum, authors should state whether extraction blanks and negative PCR controls were used, whether replicate amplifications or multi-tube approaches were applied, and whether genotyping error rates such as allelic dropout or false alleles were estimated.

Third, reports of Ho, He, and Fis should be accompanied by sufficient

methodolog- ical context to support interpretation, including marker system, number of loci or SNPs retained, filtering thresholds, missing-data criteria, and whether Hardy–Weinberg equilib- rium diagnostics and multiple-testing corrections were applied. Without this information, comparisons across studies remain difficult and potentially misleading.

Fourth, future syntheses and monitoring frameworks should treat marker systems separately where possible, because microsatellites and SNP-based approaches differ in mutation properties, allele frequency spectra, and analytical assumptions. Marker-specific reporting and synthesis will improve comparability and reduce overinterpretation of absolute metric values across technologies.

Finally, greater standardisation of reporting templates across journals, institutions, and conservation programmes would strengthen the long-term value of genetic monitoring datasets. We therefore encourage the adoption of study-level checklists that integrate sample type, DNA quality safeguards, marker characteristics, and analytical thresholds alongside the reporting of population genetic indices.

5. Conclusions

This scientometric synthesis shows that terrestrial mammal population genetics has expanded rapidly over the past four decades, with a decisive shift towards non-invasive sampling that expands taxonomic and geographic coverage while reducing animal han- dling. Crucially, once the marker system and species are taken into account, differences in Ho, He and FIS between invasive and non-invasive surveys are modest, underlining that data quality control and transparency of reporting, rather than invasiveness of sampling per se, determine the reliability and comparability of core genetic indicators.

From a methodological point of view, the transition from the microsatellite field to SNP- based, reduced representation sequencing increases resolution but complicates synthesis between studies; absolute values and variance structures differ between marker systems. Our mapping also reveals persistent regional and taxonomic biases in the research effort, along with denser international collaborative networks and an encouraging increase in open access practices that facilitate reuse and tracking. For the biological and conservation sciences, these findings have three practical implications. First, the value of non-invasive genetic data depends less on sample type itself than on rigorous quality control and transparent reporting, including replication strategies, genotyping error assessment, filtering thresholds, and diagnostics for analytical artefacts. Second, journals, societies, and conservation agencies would benefit from harmonised reporting templates that account for marker system, so that Ho, He, and Fis are interpreted more consistently across taxa and technologies. Third, strategic allocation of funding and research support to under-represented regions and taxa—together with open sharing of data, codes, and methods—would help reduce persistent knowledge gaps and strengthen conservation genetic capacity where it is most needed.

Looking ahead, integrating full-text mining of methodological details, expanding high- throughput genotyping capacity in biodiversity hotspots and jointly developing community quality checklists will improve the interoperability of genetic indicators. By harmonising ethical sampling, rigorous laboratory practices and open, standardised report- ing, conservation genetics can provide timely and comparable metrics that directly

inform species recovery and long-term population viability.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/conservation6020053/s1, S1: Total number of studies included in the scientometric analysis. S2: Summary of the 85 studies used in the analysis of population genetic indicators Ho, He and Fis.

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CHAPTER 3. NON-INVASIVE SAMPLING FOR POPULATION GENETICS OF WILD TERRESTRIAL MAMMALS (2015–2025): A SYSTEMATIC REVIEW

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Review

Non-Invasive Sampling for Population Genetics of Wild Terrestrial Mammals (2015–2025): A Systematic Review

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Abstract

Genetic variability in terrestrial mammals is essential for understanding population and evolutionary dynamics, as well as for establishing effective strategies in conservation bi- ology. This comprehensive review aimed to critically analyze invasive and non-invasive techniques used to assess genetic variability in wild terrestrial mammals. Using the PICO (Population, Intervention, Comparison, Outcome) format and following PRISMA guide- lines, a comprehensive literature search was conducted in Web of Science, Scopus and Science Direct databases, including articles published in English from January 2015 to April 2025. Thirty-one experimental studies were selected that met specific criteria related to genetic evaluation using invasive (direct blood or tissue collection) and non-invasive (stool, hair and saliva collection) techniques. The results indicate that invasive techniques provide samples of high genetic quality, albeit with important ethical and animal welfare consid- erations. In contrast, non-invasive techniques offer less disruptive methods, although they present significant challenges in terms of quantity and purity of DNA obtained, potentially affecting the accuracy and confidence of genetic analysis. Detailed analysis of selected studies showed diverse patterns of heterozygosity and inbreeding coefficients between different taxonomic orders (Carnivora, Artiodactyla, Proboscidea, Primates and Rodentia). In addition, the main anthropogenic threats and current conservation strategies implemented in different species were identified. An overall genetic variability ranging from high to moderate was observed, with large species being more vulnerable to genetic reduction due to changes in habitat and human activities. Rather than a static compar- ison, our synthesis traces a clear methodological arc from small short tandem repeats (STR, or microsatellites) panels towards SNP-based approaches enabled by next-generation sequencing, including reduced representation (ddRAD), amplicon panels (GT-seq), and hybridisation capture tailored to degraded DNA from hair, faeces, and environmental sub- strates. Over 2015–2025, study designs shifted from presence/absence and coarse diversity estimates to robust inference of relatedness, assignment, effective population size, and gene flow using hundreds–thousands of SNPs and genotype-likelihood frameworks tolerant of allelic dropout and low coverage. Laboratory practice converged on multi-tube replication, synthetic blocking oligos, and capture-based enrichment; bioinformatics adopted proba- bilistic genotype calling, error-aware filtering, and replication-based consensus. This review provides a solid basis for optimizing genetic sampling methods, allowing for more ethical and efficient studies. Furthermore, it contributes to strengthening conservation strategies by underlining the importance of adapting the sampling method to the biological and ecological particularities of each species studied. Ultimately, these findings can significantly improve genetic conservation decision-making, benefiting the sustainability and resilience of wild land mammal populations

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Keywords: biodiversity; molecular markers; biological conservation; sampling ethics

1. Introduction

Genetic variability in terrestrial mammals is a central element for understanding the ecological and evolutionary dynamics of populations, as well as for the effective implemen- tation of biological conservation measures. This variability refers to the observable genetic diversity within populations, resulting mainly from mutational processes and genetic recombination, which generate new alleles or redistribute existing ones [1]. Such processes can be significantly influenced by environmental and anthropogenic factors, which modify both the structure and long-term viability of populations [2].

Accurate estimation of genetic diversity relies in the use of advanced tools such as molecular markers including short tandem repeats (STR, or microsatellites), single nu- cleotide polymorphisms (SNPs) and copy number variants (CNVs). These tools provide detailed information on structural and point variations in DNA, allowing critical param- eters such as heterozygosity and inbreeding coefficients to be assessed [3]. High genetic diversity provides greater resilience to disease, environmental stresses and anthropogenic changes, while low diversity can compromise the survival and viability of wild popu- lations [4]. Genetic analysis in terrestrial mammals requires the collection of biological samples, which can be collected using invasive and non-invasive techniques. Invasive techniques such as direct blood or tissue collection, while providing high quality and quantity of DNA, raise important ethical and animal welfare considerations [5].

In contrast, non-invasive techniques, such as faecal, hair and saliva collection, min- imize stress on the individuals studied, although they face challenges in terms of DNA quality and quantity, which may affect the accuracy of genetic analysis [6]. In this context, the present systematic review aims to critically analyze the invasive and non-invasive techniques currently used to assess genetic variability in terrestrial mammals. Through this review we aim to clearly identify the advantages and limitations of each technique, highlighting their applicability in genetic studies and in decision-making for conservation and population management. This review will contribute significantly to the advancement of conservation biology by providing a solid basis for optimizing genetic sampling method- ologies, promoting more ethical and effective studies, and strengthening conservation strategies based on genetic science.

2. Methodology

2.1. Literature Search

In the present comprehensive review, we used the Population (P), Intervention (I), Comparison (C) and Outcome (O) format proposed by [7] to formulate the research ques- tion. Briefly, P was mammalian animals, I was genetic variability, C was the use of invasive and non-invasive techniques for tissue procurement, and O was the reported information on heterozygosity, inbreeding coefficients, and level of genetic variability in terrestrial mam- malian animals. The present systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [8]. Eligible

published scien- tific articles were identified through Web of Science, Scopus and ScienceDirect databases.

Boolean operators (AND and OR) were used to search for articles. The keyword string syntax was ("non-invasive sampling" OR "invasive sampling" OR "animal tissue") AND ("mammals" OR "wildlife" OR "wild animals") AND ("genetic variability" OR "inbreeding coefficients" OR "heterozygosity"). For up-to-date information, this systematic review only considered studies published between January 2014 and April 2025 and written in English.

2.2. Inclusion and Exclusion Criteria

Only scientific articles that met the following inclusion criteria were considered in this systematic review: (1) experimental studies assessing genetic variability in terrestrial wild mammals with invasive and non-invasive sampling; (2) articles published in English in peer-reviewed scientific journals between January 2014 and April 2025; and (3) studies providing data on genetic variability in terrestrial wild mammals with invasive and non- invasive sampling. During the study selection process, manuscripts published before 2002, not written in English, or using animals other than terrestrial free-living mammals were excluded. Duplicate papers, scientific conference abstracts, theses, books or book chapters, and traditional narrative review articles were also excluded. A total of 31 scientific articles met the inclusion criteria and were analyzed in this review (Figure 1).

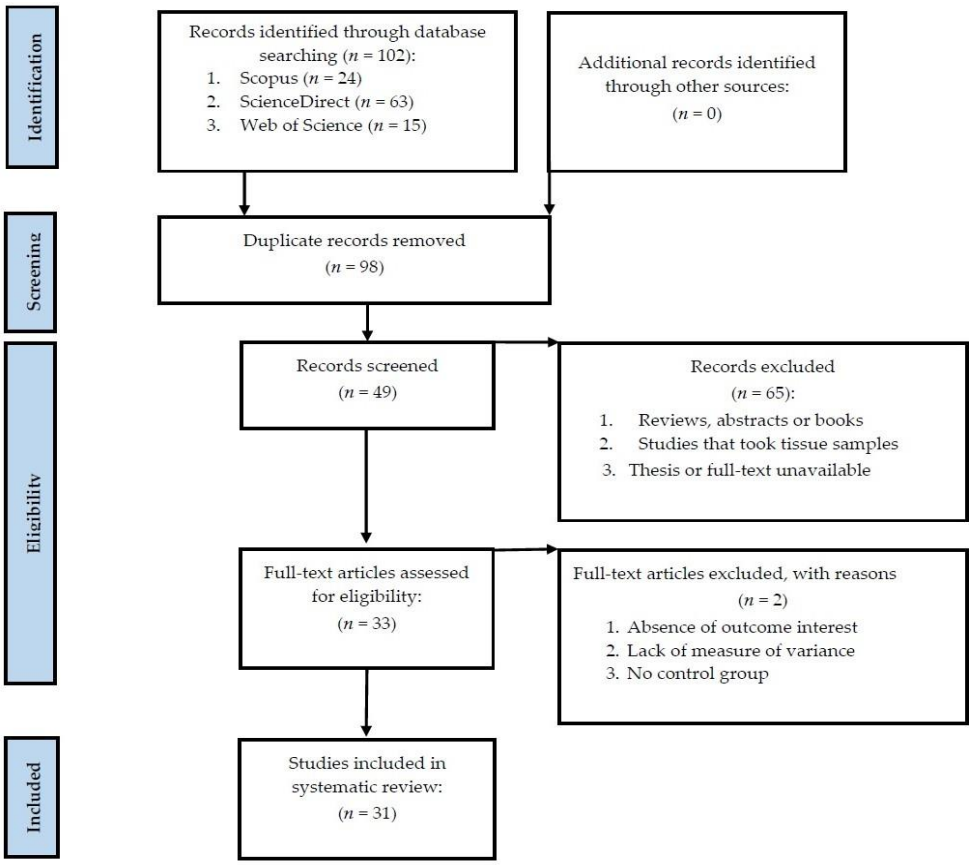


Figure 1. PRISMA flowchart showing the literature search strategy and study selection in the current systematic review.

Relevant information was extracted from each study, including the title, authors, abstract, year of publication and location of the study, and compiled into an Excel spreadsheet. The spreadsheet also included all the key data needed to address the

research question comprehensively.

3. Invasive and Non-Invasive Genetic Sampling in Terrestrial Mammals: Techniques, Challenges, and Conservation Applications

Invasive and non-invasive genetic sampling constitute the two principal methodolo- gies employed in wildlife conservation genetics (Figure 2). Each approach uses distinct techniques and therefore exhibits specific advantages and limitations that must be acknowl- edged when estimating the genetic variability of wild terrestrial mammals [9].

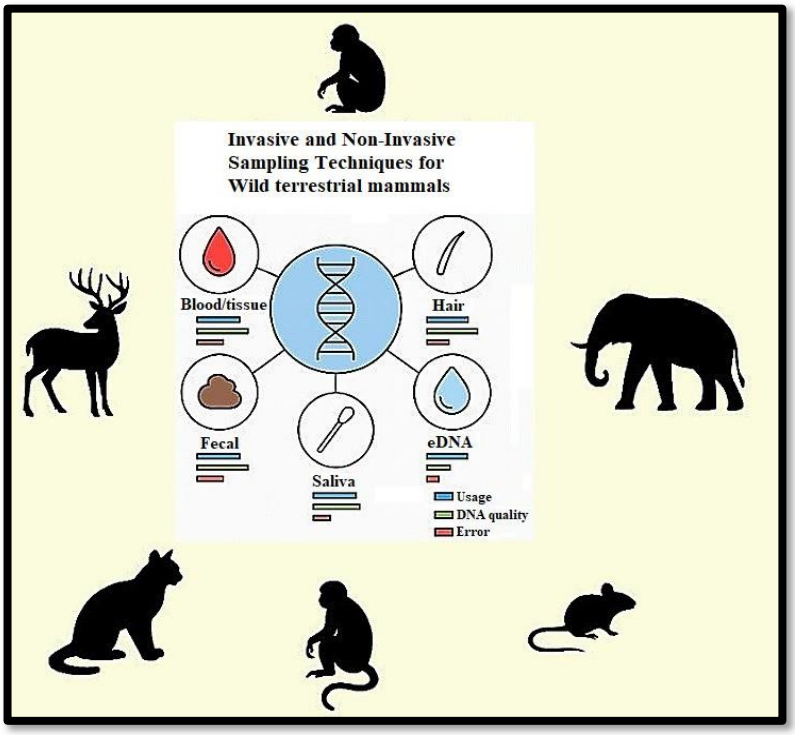


Figure 2. Key invasive and non-invasive sampling techniques, their relative usage, and indices of DNA quality and genotyping error rates.

The technique of darting has been the subject of debate in scientific discourse, with its classification as either invasive or non-invasive being contingent upon the definition of invasiveness. In the present study, darting is regarded as invasive and will not be incorporated within this category. This is in accordance with the widely accepted definition of "non-invasive" in the field of population genetics (i.e., no capture, manipulation, or skin puncture of the animal in question); remote biopsy with a dart is not non-invasive.

Historically, invasive sampling—typically involving capture, anaesthesia, and tissue biopsies—dominated conservation genetics. Although it often yields high-quality DNA, the technique is costly, labour-intensive, and can impose severe stress or injury on animals, thereby contradicting modern ethical standards that prioritise less intrusive methods [9,10]. Over the past two decades, non-invasive genetic sampling has gained prominence as an ethically preferable and logistically flexible alternative (Table 1). Faecal sampling is now the most widely applied non-invasive source because it is easy to collect, logistically inexpensive, and does not require animal handling; nevertheless, it demands multiple replicates to secure reliable genotypes owing to low DNA quantity [11].

As expected, invasive sampling achieved the highest success rate across all technolo- gies (Table 1). There was a significant improvement associated with SNP-based methods relative to STRs in non-invasive matrices, as well as a smaller but consistent advantage of invasive over non-invasive sampling. Capture- or amplicon-based SNP panels have higher initial costs but lower marginal costs when scaled up to 96–192 samples, resultingin competitive or superior costs per sample that pass quality control (QC) compared with STRs once batch sizes increase.

Table 1. Summary of published studies in which both non-invasive and invasive genetic sampling were conducted in terrestrial mammals.

Species	N	n of Non-Invasive Samples	n of Invasive Samples	Type of Marker	Reference
Otocolobus manul	16	6	10	STR,12S ribosomal	[12]
Lynx lynx	6	5	1	STR	[13]
Puma concolor	3355	0	3355	STR	[14]
Ursus arctos	44	27	17	STR	[15]
Lutra lutra	47	47	0	STR, mitochondrial cytochrome b gene	[16]
Panthera uncia	213	213	0	STR, mitochondrial cytochrome b gene	[17]
Leopardus geoffroyi	172	163	9	STR	[18]
Mellivora capensis	194	113	81	STR	[19]
Panthera onca	536	536	0	STR	[20]
Panthera tigris	718	718	0	STR	[21]
Vulpes vulpes	150	150	0	STR	[22]
Felis nigripes	23	23	0	STR, mithochondrial DNA ND5	[23]
Panthera tigris tigris	71	12	59	STR	[24]
Alces alces	1058	529	529	STR	[25]
Ozotoceros bezoarticus	155	142	13	STR, mithochondrial DNA	[26]
Odocoileus hemionus fuliginatus	238	238	0	STR	[27]
Cervus hanglu hanglu	160	160	0	STR, mitochondrial D-loop	[28]
Elephas maximus	252	252	0	STR, mitochondrial DNA	[29]
Alouatta caraya	138	132	6	STR, mitochondrial DNA	[30]
Sciurus griseus	117	114	3	STR, mitochondrial DNA D-loop	[31]
Hydrochoerus hydrochaeris	54	27	27	STR, mitochondrial DNA	[32]
Loxodonta africana	37	37	0	Mitochondrial genomes	[33]
Tremarctos ornatus	38	38	0	STR, mitochondrial D-loop	[34]

Hair represents another common non-invasive substrate that generally contains higher endogenous DNA concentrations than faeces; however, retrieving and amplifying hair DNA still calls for careful extraction protocols to minimise allelic dropout and genotyping errors [35]. Saliva and chewed plant material offer unique, minimally disruptive opportuni- ties for collecting buccal epithelial cells that supply high-quality nuclear DNA; nevertheless, stringent field and laboratory protocols are required to prevent contamination and DNA degradation when using these matrices in population-genetic analyses [33].

Environmental DNA (eDNA) has emerged as a cutting-edge methodology in con-servation genetics. By analysing the DNA shed into soils, sediments or bodies of water, researchers can infer the presence of species and genetic diversity without direct observa- tion, provided rigorous contamination control and taxonomic verification standards are met [36,37]. The preservation of collected samples is critical, particularly for

non-invasive materials which often contain fragmented or inhibitor-laden DNA. Environmental DNA (eDNA) metabarcoding is a non-invasive method of surveying biodiversity that analyses extracellular and shed DNA present in environmental samples (e.g., water, soil, air). After sampling, the DNA is extracted, and short taxonomically informative loci (commonly the mitochondrial 12S or COI gene for animals and the 16S gene for prokaryotes) are amplified by PCR and sequenced using high-throughput platforms. Bioinformatic pipelines then assign sequences to taxa using curated reference databases, producing multi-species inven- tories from a single sample. Silica gel desiccation, cold-chain transport, and the addition of chelating buffers remain the most reliable strategies for retaining genetic integrity and thereby reducing downstream analytical error. When properly preserved, non-invasive substrates permit the successful amplification of both mitochondrial (mtDNA) and nuclear (nDNA) markers, each providing complementary information on matrilineal history and contemporary gene flow, respectively [9]. Mitochondrial DNA is frequently chosen because of its high copy number per cell, rapid evolutionary rate, and maternal mode of inheritance, which collectively facilitate inferences on phylogeography and historical demography in mammalian populations [38,39].

Among nuclear loci, STR remain the most widely applied markers in conservation-genetic studies owing to their high polymorphism and codominant inheritance. STR panels developed for carnivores and ungulates have revealed substantial genetic diversity and have served to identify incipient inbreeding in isolated populations. Sex-linked markers—in particular those located on the Y-chromosome and the X-inactivation centre—are essen- tial for diagnosing sex ratio, paternity, and dispersal patterns, facilitating the design of sex-specific management interventions [40]. Despite these advantages, molecular markers derived from non-invasive samples are susceptible to genotyping errors caused by allelic dropout, false alleles, and heterozygote deficiency, all of which can bias population-genetic parameters if left uncorrected [41]. DNA degradation constitutes a major challenge because environmental exposure accelerates depurination and oxidative damage, producing short fragments that are difficult to amplify. Carnivore faeces often contain higher concentra- tions of digestive inhibitors than herbivore dung owing to their protein-rich diet and gut physiology [42].

Low DNA yield represents a further constraint, particularly when studying elusive or endangered species with inherently small population sizes. Studies on felids and mustelids have routinely reported amplification failure rates exceeding 50% due to marginal DNA quality and quantity [43]. Plant secondary metabolites, humic acids, and bile salts act as potent polymerase inhibitors in faecal DNA extracts, significantly diminishing amplifi- cation efficiency [44]. Several technical solutions have been proposed to improve DNA recovery from challenging substrates, including the adoption of combination extraction kits, inhibitor-binding resins, and low-temperature lysis protocols, all of which have markedly enhanced genotyping success [45]. External PCR controls, allele-specific replication, and the adoption of multi-tube consensus approaches effectively mitigate amplification artefacts associated with fragmented DNA templates [46]. High-throughput sequencing technology, coupled with rigorous replication and stochastic error-modelling frameworks, has substan- tially reduced genotyping error rates and improved individual identification accuracy [9,35].

In summary, although non-invasive genetic sampling introduces technical obstacles,

recent methodological advances have minimized these limitations, making the approach a robust, ethical, and cost-effective tool for monitoring genetic diversity, population structure, and demographic trends in wild terrestrial mammals, thereby providing indispensable data for evidence-based conservation.

4. Genetic Variability in the Order Carnivora

4.1. Main Species and Their Heterozygosity Values

The Carnivoraformes clade comprises mammals such as tigers, lions, bears, raccoons and others including their ancestors, distinguished by their diet and jaw structure as mesocarnivores (50% to 70% animal matter), hypocarnivores (<50% animal material), hypercarnivorous (>70% animal matter) or bone breakers [47]. At least for this group, studies of autosomal heterozygosity are confirmed in felids such as Iberian bobcats, pumas, cheetahs, jaguars, lions and leopards [48]. The trend in their genetic diversity is related to the dimensionality of their populations for microsatellite studies [49]. In addition, it is known that the decline in their populations causes high inbreeding values, reducing the gene pool of the species, mainly due to anthropogenic causes [50]. In this regard, Table 2 shows studies related to expected and observed heterozygosity in the order Carnivora and their inbreeding pairings, where it is indicated that when the value of Ho is less than He, it is possible that more inbred matings exist. In addition, each of the threats to the populations and their implemented conservation strategies are described. Furthermore, their genetic variability status according to the authors is included, using expected heterozygosity (He) thresholds to categorize diversity as low, moderate, or high.

Table 2. Main species studied and their genetic heterogeneity values in the order Carnivora

Species	Main Threats	Conservation Strategy	Type of Sample	Expected and Observed Heterocigosity *	Reference
Otocolobus manul	Habitat degradation and climate change	Poaching mitigation, creation of NPAs and Captive Areas	Faeces and blood.	He = 0.62 Ho = 0.57	[12]
Lynx lynx	Poaching and poor habitat quality	Reintroduction	Faeces, blood and tissue	-	[13]
Puma concolor	Urbanisation and habitat loss and fragmentation	Protected natural areas	Skin and muscle	He = 0.59 Ho = 0.52	[14]
Ursus arctos arctos	Human activities	Reintroduction	Blood, tissue, excreta and hair	He = 0.61 Ho = 0.64	[15]
Ursus arctos marsicanus	Human activities	Reintroduction	Blood, tissue, excreta and hair	He = 0.40 Ho = 0.39	[15]
Lutra lutra	Habitat fragmentation	Reintroduction	Faeces	Ho = 0.37	[16]

Panthera uncia	Habitat fragmentation	Translocation	Faeces	He = 0.57 Ho = 0.54	[17]
Leopardus geoffroyi	Habitat fragmentation and land use change	Biological corridors	Tissue and blood	He = 0.77 Ho = 0.70	[18]
Meles meles	Urbanisation	Biological corridors	Fresh, dead tissue and faeces	He = 0.45 Ho = 0.42	[19]
Panthera onca	Habitat fragmentation	Biological corridors and protected areas	Faeces	He = 0.61 Ho = 0.55	[20]
Panthera tigris tigris	Poaching and habitat loss	Biological corridors	Faeces	He = 0.77 Ho = 0.68	[21]
Vulpes vulpes	Urbanisation and population isolation	-	Faeces	He = 0.55 Ho = 0.52	[22]
Felis nigripes	Overgrazing and extensive agriculture	Captive areas	Hair	He = 0.62 Ho = 0.68	[52]
Panthera tigris tigris	Poaching and habitat loss	Biological corridors	Blood and excreta	He = 0.64 Ho = 0.50	[24]

4.2. Population Balance and Inbreeding Coefficients

The Hardy–Weinberg equilibrium is commonly used for population analysis in mam- mals as an input to genetic analyses using STR that can be performed in free-living and captive species. STR can also be used to identify null alleles and heterozygosity deficits in subpopulations, providing the framework for evaluating deviations from equilibrium and quantifying Wright's F-statistics [14,24]. In populations, loci in Hardy–Weinberg equilib- rium have heterozygosity levels that conform to expected values, but inbreeding coefficient Fis varies from −1 to +1 values. For example, in the study conducted on the species *Oto- colobus manul*, genetic diversity values ranged from low to moderate levels with Fis values ranging from −0.093 to 0.105 for zoo-managed and wild populations, respectively [12]. In another similar case in *Puma concolor*, almost all loci were under the null expectation of HWE, with an observed heterozygosity significantly lower than expected heterozygosity and Fis ranging from 0.05 to 0.12 with a mean of 0.07; *Felis nigripes* was estimated at 0.048, both with moderate diversity [14,23]. However, an imbalance in the equilibrium can reveal biological and ecological patterns, as in the case of *Vulpes vulpes* where there was HWE imbalance indicating a reduced number of individuals, absence of random mating, and inbreeding, with an approximate value of Fis = 0.047 [22]. A similar pattern was observed in the case of *Lynx lynx*, where genetic differentiation among populations had lower values Fst = 0.097 [13].

Positive Fis values also herald a deviation in HWE and heterozygote deficiency,

as was the case for *Meles meles*, with a value at Fis = 0.021 [19]. Furthermore, deviation from equilibrium can be significant in some species that maintain stable genetic variability in their populations; this apparent contradiction arises when the expected heterozygosity (He) remains moderate or high while observed heterozygosity (Ho) does not, such as in the cases of *Leopardus geoffroyi*, with a population value of Fis = 0.093, and *Panthera leo*, Fis = 0.03 [18,51]. It is possible to have this value in a single locus, as suggested by [21] in the species *Panthera tigris tigris* with an estimated value Fis = 0.208.

In summary, based on available data, certain species within the carnivorous order can maintain high-moderate variability with different mammal conservation strategies, mostly reintroduction programmes, biological corridors and establishment of natural protected areas. Anthropogenic adversities have threatened some species; however, those that maintain moderate variability are latently deprived if more conservation measures appropriate to each situation are not implemented. Most of the genetic studies were carried out with STR considering the Hardy–Weinberg equili

law, estimating observed and expected heterozygosity as well as inbre
coefficients, and were based mostly on DNA extracted from invasive and noninvasive samples. Such tools are fundamental for decision-making in carnivore conservation in different parts of the world to increase species variability and coexistence of populations.

5. Genetic Variability in the Order Artiodactyla

5.1. Main Species and Their Heterozygosity Values

The main characteristic of artiodactyls is that they have a pair of toes or digits, like ungulate mammals and other paraxonians [52]. In this order, genetic variability studies have been carried out measuring heterozygosity [53]. Some authors consider that the size of larger and more mobile mammals has lower levels of genetic variability than that of smaller and less mobile mammals, which implies the search for new habitat territories [54], hence the importance of estimating their variation. Table 3 shows the expected and observed heterozygosity values, threats and conservation strategies for 10 species belonging to the order Artiodactyla.

5.2. Population Balance and Inbreeding Coefficients

Different studies on ungulates estimated the Hardy–Weinberg equilibrium and in- breeding coefficients. For instance, the species *Ozotoceros bezoarticus* had an estimated Fis coefficient of 0.157, which could suggest a slight difference in heterozygotes and indicate a certain level of inbreeding [26]. Similarly, in the species *Cervus elaphus hanglu*, which experienced a genetic bottleneck that altered mutations and provided high heterozygosity values with respect to HWE equilibrium, the Fis coefficient was estimated at 0.38 [28]. By contrast, population deviation in *Sus scrofa* indicated the Wahlund effect, whereby the population substructure prevented free mating and reduced heterozygosity, with Fis estimated at 0.051 [55].

Table 3. Main species studied and their heterogeneity values in the order Artiodactyla.

Species	Main Threats	Conservation Strategy	Genetic Sampling	Expected and Observed Heterocigosity	Reference
Ammotragus lervia	Illegal hunting	Conservation and translocation management units	Faeces, hair, bone and tissue	He = 0.49 Ho = 0.43	[57]
Ammotragus lervia	Poaching and habitat loss	None	Blood and tissue	He = 0.48 Ho = 0.46	[58]
Alces americanus americanus	Physical and anthropogenic barriers	Connectivity between towns	Hair and fabric	He = 0.32 Ho = 0.35	[25]
Sus scrofa	Habitat reduction	Urban colonisation	Muscle and tissue	He = 0.59 Ho = 0.56	[56]
Naemorhedus griseus	Consanguinity	Species in captivity for reintroduction	Blood	He = 0.45 Ho = 0.19	[59]
Lama glama	Species utilisation	Species in captivity for reintroduction	Blood	He = 0.76 Ho = 0.71	[60]
Camelus dromedarius	None	Research only	Blood	He = 0.72 Ho = 0.67	[61]
Ozotoceros bezoarticus	Agriculture and urbanisation	Population genetic variability studies	Faeces	He = 0.75 Ho = 0.72	[26]
Tragelaphus eurycerus ssp. isaaci	Human activities	Species in captivity for reintroduction	Faeces and tissue	He = 0.42 Ho = 0.71	[62]
Odocoileus hemionus fuliginatus	Fragmentation of habitat and urbanisation	Natural protected areas and biological corridors	Faeces	He = 0.56 Ho = 0.56	[27]
Cervus elaphus hanglu	Habitat reduction	Protected natural areas	Hair	He = 0.66 Ho = 0.44	[28]

In particular, the order Artiodactyla is considered important because of the different uses of the species that comprise it, most of which are used for poaching, which if controlled legally does not affect the populations; however, when there is no control, the populations tend to decline, and this affects the genetic variability of the species. It is worth mentioning that some species exhibit invasive behaviour that may affect other species in their niche; despite this, most are in some category of threat. Compared to other orders, studies were mostly carried out with invasive samples and a minority with faecal samples; this encouraged the advancement of genetic sampling and heterozygosity–endogamy analysis for populations. In general, the order maintains moderate-low genetic variability, due to anthropogenic causes.

6. Genetic Variability in the Orders Proboscidea, Primates and Rodentia

6.1. Main Species and Their Expected and Observed Heterozygosity Values

The order Proboscidea comprises large mammals such as living and extinct elephants (*Elephantidae*), characterised by the proboscis (trunk), which is a combination of nose and upper lip, and enlarged tusks. Genetic variability studies have differentiated elephant species, adding that species in a forested habitat are more variable than in the African savannah [62]. In another case, the order Primates is divided into four genera Homo (Humans), Simia (monkeys and thirds), Lemur (lemurs and lorises) and Vespertilio. The main characteristics of the order lie in the upper front teeth and two mammary glands on their pectorals [63]. For studies of their genetic variability, population genetic analyses

are available for the species *Alouatta caraya* of Argentina, Paraguay and Brazil [30]. The situation is different for the order Rodentia, considered the most diverse among mammals, characterised by a gnawing dentition composed of a pair of lower incisors with continuous growth [64]. Studies on genetic variability have prioritised their high variation due to environmental homogeneity, in addition to very little inbreeding in their populations [65]. For these orders, Table 4 describes the species of each order, as well as their heterozygosity values, problems and conservation strategies in each of the populations studied from a genetic point of view.

Table 4. Main species studied and their heterogeneity values in the orders Proboscidea, Primates and Rodentia.

Species	Main Threats	Conservation Strategy	Genetic Sampling	Expected and Observed Heterocigosity	Reference
Elephas maximus	Habitat fragmentation and reduced gene flow	Semi-captive species	Blood	He = 0.64 Ho = 0.63	[65]
Elephas maximus	Illegal hunting	Semi-captive species	Faeces	He = 0.67 Ho = 0.57	[29]
Alouatta caraya	Deforestation, agriculture and livestock	Reintroduction and management units	Faeces and tissue	He = 0.44 Ho = 0.46	[30]
Sciurus griseus *	Habitat loss and fragmentation due to urbanization	-	Hair	He = 0.49 Ho = 0.45	[31]

6.2. Population Balance and Inbreeding Coefficients

For the order Proboscidea, population variability was high in both studies, with expected heterozygosis (He) values ranging from 0.21 to 0.90 according to the Hardy–Weinberg equilibrium hypothesis in *Elephas maximus* specimens [29]. However, these investigations did not consider the calculation of inbreeding coefficients. In the order Primates for the species *Alouatta caraya*, variability was high with a negative inbreeding coefficient (Fis = −0.06), but there was no population imbalance [30]. In the case of rodents of *Sciurus griseus*, the deviation was significant but the variability was moderate and there was a value of Fis = −0.018 [31]. The orders analyzed in this study are sparser and are different from the others mentioned above. However, small mammals are not so affected and their population variability and viability is compromising for their subsistence. It is worth mentioning that the estimation of inbreeding coefficients can help all three orders, understanding that it is a useful technique for any type of mammal. The samples analyzed were mostly hair samples. However, few studies are considered for the very large orders.

7. 2015–2025: Methodological Shifts and Best Practices in Non-Invasive Population Genomics

In 2015–2018, reduced-representation methods (RAD and ddRAD) were piloted on non-invasive DNA but suffered from allele dropout and locus dropout due to fragmentation and inhibitors. From ~2018 onward, two strategies rose to prominence for degraded templates: amplicon panels (GT-seq)—hundreds of short (<120–150 bp) targets multiplexed per sample and hybridisation capture of short SNP-bearing fragments using custom baits, often with single- or unique-molecular-index (UMI) libraries to track duplicates. These approaches improved locus recovery and cross-study comparability

while keeping per- sample costs manageable. STRs remain useful for continuity with legacy datasets and for certain kinship applications, but SNP panels now dominate where non-invasive sampling is routine.

Early non-invasive studies emphasised detection, minimum population size, or broad diversity (He) and FIS estimates, constrained by 8–20 STR loci and genotyping error. With hundreds–thousands of SNPs, 2019–2025 studies routinely estimated relatedness, assignment, hybridisation, and effective population size with tighter precision, and detected fine-scale structure and sex-biased dispersal. Demographic inference shifted from methods requiring long contiguous haplotypes (unsuitable for fragmented DNA) to site–frequency– spectrum and genotype likelihood approaches that tolerate low coverage. Kinship and assignment accuracy improved notably as panels incorporated ancestry-informative and immune-linked SNPs while controlling for missingness.

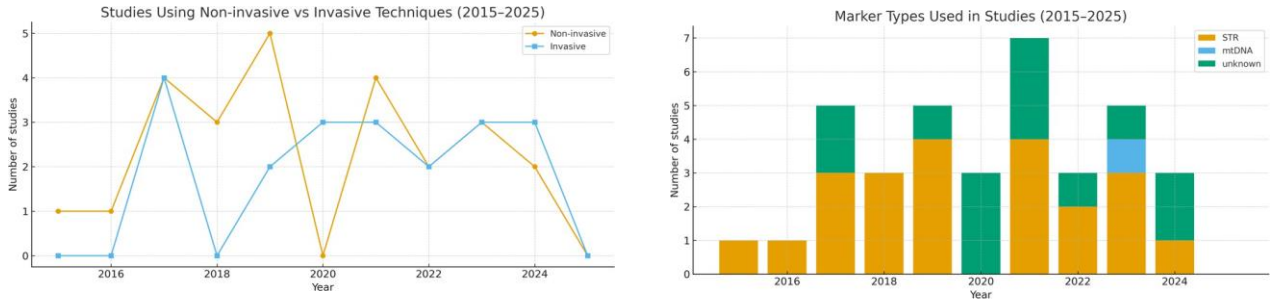
Best practice converged on (1) multi-tube replication at extraction and PCR to quantify allelic dropout; (2) small-amplicon design (<120 bp) or capture of short inserts; (3) blocking oligos or predator-specific PNA and LNA clamps to reduce non-target amplification in faecal samples; (4) UDG treatment when deamination patterns suggest ancient-DNA- like damage; (5) consistent use of UMIs and dual indices to control index hopping; and (6) rigorous negative controls and extraction blanks. Field preservatives shifted from vari- able ethanol/silica protocols to validated buffers (e.g., Longmire's or equivalent commercial stabilisers) with documented inhibitor removal at extraction. The period saw a transition from hard genotype calls to probabilistic genotype- likelihood frameworks that explicitly model dropout and depth (e.g., ANGSD-like pipelines), followed by error-aware filtering (allele balance, strand bias, depth bounds) and replication-informed consensus. For amplicon and capture panels, per-locus performance metrics (on-target rate, duplicate fraction, allelic dropout rate) are now reported alongside population parameters, improving reproducibility and meta-analysis.

While shotgun sequencing remains inefficient for low endogenous DNA, target capture reduced sequencing waste and made SNP-scale inference feasible for programs with modest budgets. Amplicon panels require upfront design but enable scalable monitoring. Method selection increasingly reflects decision frameworks balancing aims (assignment vs. Ne), sample type (hair vs. faeces), DNA quality, and resources.

Terrestrial eDNA and metabarcoding matured for detection and community composi- tion (soil, leeches, carrion flies), but only rarely support population-genomic inference at present. Our synthesis positions eDNA as complementary for occurrence and surveillance, while individual-assignable non-invasive samples (scat/hair/urine) remain the backbone for population genetics. The last decade normalised explicit reporting of permits, animal welfare minimisation, and FAIR data deposition of both raw reads and panel definitions (probe/amplicon lists), enabling re-use and cumulative gains.

Across 2015–2025, non-invasive applications (including "both") increased steadily and consistently outnumbered invasive-only studies in recent years, reflecting the shift toward minimally disruptive sampling at scale. Marker usage was dominated by STR and mtDNA early in the period, with an evident rise of SNP/NGS-

oriented approaches toward the latter years, consistent with the review's narrative on the transition from small STR panels to SNP-based genotyping suitable for degraded templates. Taken together, the timeline highlights the field's methodological drift toward ethical, logistically scalable sampling coupled with higher-resolution markers, aligning population-genetic inference with contemporary conservation needs (Figure 3).



(**A**) (**B**)

Figure 3. (**A**) Studies using non-invasive versus invasive techniques per year (2015–2025); (**B**) marker types used per year. Values reflect counts of unique studies per calendar year; studies classified as "both" contribute to both non-invasive and invasive tallies. Notes: Technique categories—non- invasive (e.g., faeces/scat, hair, saliva/chews, urine, environmental DNA), invasive (e.g., blood, tissue, muscle, skin, bone, biopsy), and "both" when a study reported samples from both groups. Marker categories—STR (microsatellites), mtDNA (e.g., COI, cytochrome b, D-loop, 12S), SNP/NGS (e.g., SNP panels, ddRAD/RAD, GT-seq, hybrid capture), and other/unspecified for markers not mapping to the above.

8. Future Orientations

Future research should focus on improving the quality and quantity of DNA obtained by non-invasive methods, optimizing specific molecular protocols for each type of biological sample. Further comparative studies between different taxonomic orders are also needed to generalize results and recommendations. It is recommended to explore new molecular techniques such as massive sequencing and advanced bioinformatics methods that can compensate for the limitations on the quality of DNA obtained from non-invasive methods. Further research on the ethical impact and social perceptions related to the use of invasive methods in different cultural and social contexts would also be beneficial.

9. Limitations of the Study

An important limitation of this review was the restriction to studies published in En- glish only, which could exclude relevant research published in other languages. In addition, the methodological heterogeneity observed among the reviewed studies limited direct comparison of results, making it difficult to generalize definitive conclusions. Another limitation is the absence of specific analysis on the direct environmental impact associated with each sampling method, which could be relevant for future research.

10. Conclusions

This systematic review collated data from 31 peer-reviewed studies, published between 2015 and April 2025, which compared the effectiveness of invasive versus non-invasive genetic sampling techniques in wild terrestrial mammals. While invasive sampling consis- tently yielded the highest DNA integrity and facilitated genomic analyses, it necessitated the capture and anaesthesia of animals, thereby increasing costs and risks to animal welfare. Conversely, non-invasive methodologies reduced disruption and facilitated population- scale coverage; nevertheless, they were plagued by issues including diminished DNA quality, allele loss, and contamination. The concept of environmental DNA (eDNA) repre- sents a novel methodology that facilitates the estimation of presence/absence and diversity without the necessity for direct contact. Conversely, recent advances in conservation techniques (e.g., silica desiccation, cold chain, chelating buffers) now enable the reliable am- plification of DNA from degraded sources. Nevertheless, the phenomenon of marker bias remains in evidence. Despite the existence of SNP and CNV platforms, STR predominate. The statistical robustness of these methods is contingent upon the implementation of cor- rections for allele loss and Hardy–Weinberg deviations. Studies that employed multitube consensus attained reduced genotyping error rates.

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CHAPTER 4. FECAL MICROBIOME OF WILD AND DOMESTIC RUMINANTS IN SYMPATRY: A COMPARATIVE ANALYSIS BETWEEN *Ammotragus lervia*, B*os taurus,* AND *Capra aegagrus hircus* IN AN ARID ECOSYSTEM OF THE CHIHUAHUA DESERT

Abstract

The gut microbiome plays a fundamental role in the physiology, nutrition, and ecological adaptation of ruminants, particularly in extreme environments such as arid ecosystems. In this study, the fecal microbiome of the Barbary sheep (Ammotragus lervia), an introduced wild species, with that of domestic ruminants—cattle (Bos taurus) and goats (Capra aegagrus hircus)—under conditions of sympatry in the Cañón de Santa Elena Flora and Fauna Protection Area, in the Chihuahuan Desert, Mexico. A total of 15 fecal samples were analyzed using V3–V4 region sequencing of the 16S rRNA gene and a bioinformatics pipeline based on QIIME2 and DADA2. The results revealed that the three species share a similar taxonomic structure at the phylum level, dominated by Firmicutes and Bacteroidota, suggesting microbial convergence associated with shared resource use and environmental conditions. However, beta diversity analyses (Jaccard, Bray–Curtis, and UniFrac) showed significant differences between species ($p < 0.05$), indicating a divergence in microbial composition influenced by host identity. Alpha diversity did not show significant differences in richness and evenness indices, although Faith's phylogenetic diversity was higher in cattle. The SIMPER analysis identified key taxa responsible for the dissimilarity between species, highlighting genera associated with fiber fermentation. Taken together, these results indicate that sympatry generates complex patterns of microbial convergence and divergence in ruminants. This study contributes to the understanding of microbiome ecology in arid systems and provides relevant information for wildlife management, animal production, and health under a One Health approach.

Keywords: Gut microbiome, Sympatrous ruminants, Arid ecosystems, *Ammotragus lervia*

1. INTRODUCTION

The gut microbiome of vertebrates constitutes a complex and dynamic system that performs essential functions in the host's digestion, metabolism, and immune regulation (Berg et al., 2020). In ruminants, these microbial communities are particularly relevant due to their role in the fermentation of structural polysaccharides and in energy utilization efficiency, which directly impacts ecological adaptation and productive performance (Henderson et al., 2015). In this context, the analysis of the fecal microbiome using next-generation sequencing technologies has enabled advances in the characterization of these communities and in understanding the factors that determine their structure and function (Bolyen et al., 2019).

Several studies have shown that the composition of the gut microbiome in ruminants is strongly influenced by factors such as diet, environment, management, and the host's physiological state (Henderson et al., 2015; Reese et al., 2021). Likewise, domestication has been identified as a process that alters microbial diversity and composition, generally reducing the variability observed in wild populations (Reese et al., 2021). These differences may have important implications for the ecological resilience, health, and metabolic efficiency of animals.

In ecosystems where wild and domesticated species coexist, sympatry creates opportunities for the exchange of microorganisms and the functional convergence of their microbiomes, particularly when there is overlap in the use of food resources (Karmacharya et al., 2024). This phenomenon has been associated not only with changes in microbial diversity but also with the potential transfer of antimicrobial resistance genes, which raises relevant implications from a One Health perspective (Al-Khalaifah et al., 2025). However, the magnitude and direction of these processes depend on multiple ecological factors, including the intensity of interspecies interaction and environmental conditions.

The Barbary sheep (Ammotragus lervia) is an exotic species introduced into various arid regions, where it has established feral populations that coexist with domestic livestock (Castellanos et al., 2019). In the Cañón de Santa Elena Flora and Fauna Protection Area, in the Chihuahuan Desert, this species shares habitat with cattle (*Bos taurus*) and goats (*Capra aegagrus hircus*) under extensive grazing systems. This coexistence raises relevant questions regarding ecological competition, shared resource use, and potential effects on the structure of the gut microbiome.

Despite growing interest in microbiome ecology, there is a clear gap in the literature regarding studies that simultaneously compare the fecal microbiomes of wild and domestic ruminants under sympatric conditions within arid ecosystems. Most available studies have focused on domestic species under controlled conditions or on wildlife in isolation, limiting our understanding of microbial interaction processes in complex natural systems (Henderson et al., 2015; Reese et al., 2021). In particular, no studies have been documented that integrate the

Barbary sheep with domestic species in the Chihuahuan Desert from a comparative microbiological perspective.

Therefore, the present study aims to characterize and compare the fecal microbiome of *Ammotragus lervia*, *Bos taurus*, and *Capra aegagrus hircus* under conditions of sympatry, using sequencing of the V3–V4 region of the 16S rRNA gene. It is hypothesized that the ecological overlap among these species could be reflected in patterns of convergence and divergence in the structure of their microbial communities. This approach will not only expand knowledge of the microbial ecology of ruminants in arid environments but also provide relevant evidence for wildlife management and the sustainability of production systems in desert regions.

2. MATERIALS AND METHODS

2.1 Study Area

The APFF covers a total area of 277,209.00 hectares located in the municipalities of Manuel Benavides and Ojinaga, Chihuahua (Figure 1). The climate in the region is arid dry (type BWhw) according to the Koppen classification. The average annual temperature ranges from 18 to 22 °C, and average annual precipitation ranges from 286.1 to 570.71 mm. The vegetation composition consists of four main plant communities: riparian vegetation, desert scrub (microphyllous and rosette-forming), grassland, and forest.

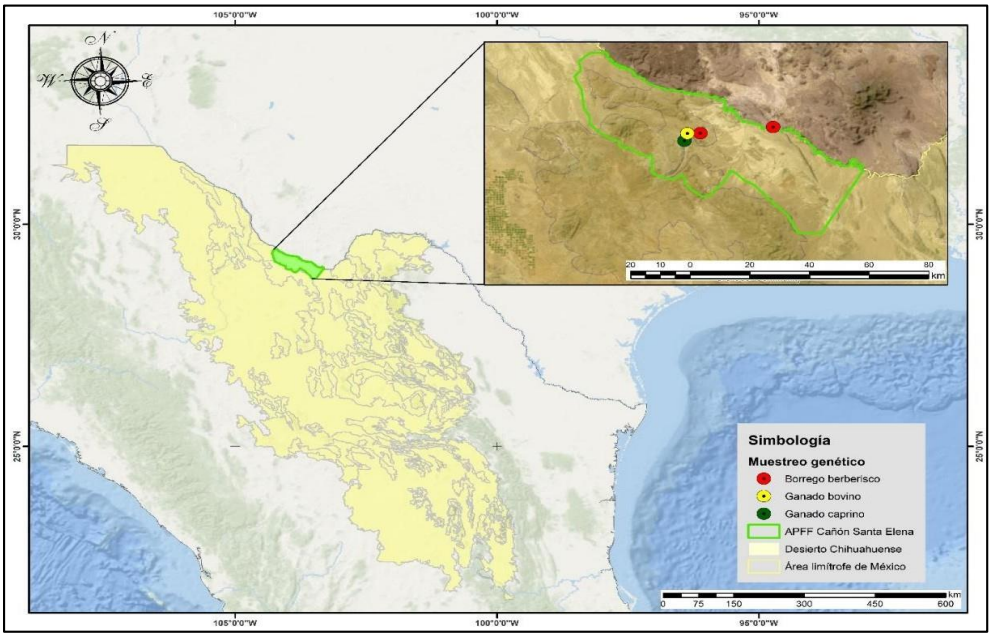


Figure 1. Santa Elena Canyon Flora Protection Area. The collection of fecal samples from Barbary sheep is shown in red, and the sampling locations for domestic cattle are shown in green and yellow.

2.2. Fecal Sample Collection

In July 2025, a total of 15 fecal samples were collected. Five from Barbary sheep, five from cattle, and five from goats. The sampled individuals were randomly selected from their respective flocks and herds. In the case of Barbary sheep, these were culled in accordance with the control program within the Santa Elena Canyon Flora Protection Area, and samples were taken from the distal portion of the animal's large intestine. For cattle sampling, samples were taken in the holding pens directly from the animal's rectum. Approximately 100 g of feces was collected from each animal and refrigerated at 5 °C for transport and subsequent analysis. Immediately upon arrival at the laboratory, 1 cm^3 of the sample was extracted and placed in a tube with lysis beads (Bashing Beads) containing 750 µL of lysis/stabilization solution, where it was stored for subsequent DNA extraction.

2.3 Laboratory processing and analysis

Bacterial DNA was extracted from fecal samples using the ZymoBIOMICS™ DNA MiniPrep kit (Zymo Research, Irvine, CA, USA), following the manufacturer's instructions. All laboratory procedures were performed in a laminar flow hood that had been previously sterilized with ultraviolet light to minimize the risk of contamination. The extracted DNA was then sent to Novogene Corporation, Inc. (Davis, CA, USA), where amplification and sequencing of the V3–V4 region of the 16S rRNA gene were performed using the universal primers 341F (CCTAYGGGRBGCASCAG) and 806R (GGACTACNNGGTATCTAAT).

The PCR (Polymerase Chain Reaction) reactions were prepared in a final volume of 15 µL, using the Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, USA), with 2 µM of each primer and approximately 10 ng of template DNA. The amplification program (thermal cycles) included an initial denaturation at 98 °C for 1 minute, followed by 30 cycles with the following steps: denaturation at 98 °C for 10 seconds, annealing at 50 °C for 30 seconds, and extension at 72 °C for 30 seconds. The resulting products were verified by 2% agarose gel electrophoresis, stained with SYBR™ Green (Thermo Scientific, Waltham, MA, USA). Subsequently, the DNA fragments (amplicons) were mixed in equal (equimolar) proportions and purified using the QIAquick Gel Extraction Kit (Qiagen™, Hilden, Germany). Sequencing libraries were prepared using the TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, San Diego, CA, USA), incorporating indices to identify each sample. The quality and concentration of these libraries were assessed using a Qubit® 2.0 fluorometer (Thermo Scientific) and an Agilent Bioanalyzer 2100 system (Agilent Technologies, Santa Clara, CA, USA). Finally, sequencing was performed on the Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA), generating paired-end reads of 250 base pairs. It is important to note that this study did not include negative extraction or PCR controls; therefore, no bioinformatic methods were applied for the detection and removal of contaminants. Consequently, the results were interpreted with caution, taking this limitation into account (Cortinas-Salazar et al., 2026).

2.4 Bioinformatic Analysis

Bioinformatic analysis of the sequences was performed using the QIIME2 (Quantitative Insights into Microbial Ecology) software on the Linux Ubuntu platform (Bolyen et al., 2019). For data removal and cleaning, the DADA2 algorithm (divisive amplicon noise removal algorithm) was employed. This allowed for the removal of low-quality sequences and the filtering of chimeric sequences, as well as the generation of amplicon sequence variants (ASVs) (Callahan et al., 2016). Good's coverage (Good, 1953) was estimated for each sample to determine whether the sequencing depth was sufficient; values between 0.95 and 1.00 indicated adequate coverage. Taxonomic assignment was performed using the Greengenes2 database (McDonald et al., 2024).

Subsequently, the species-level ASVs in the data repository available at NCBI (National Center for Biotechnology Information) were verified using the BLAST (Basic Local Alignment Search Tool). To be considered a valid species-level assignment, three criteria had to be met: (1) an identity percentage greater than 98.0%, with no equivalent matches to other related taxa; (2) the E-value had to be equal to or less than 0.0; and (3) an alignment coverage (query coverage) of 100%. For those taxa where any of these conditions were not met, the taxonomic classification was maintained at the genus level. Reaching the species level (Latinized name) was considered relevant because the precise taxonomic identification of the bacteria circulating in the blood of cows, goats, and Barbary sheep is key to understanding their function in each of the analyzed organisms.

The relative abundance of the predominant ASVs at the phylum, family, and species levels in the three species was presented graphically using heatmaps in the Morpheus program (Broad Institute). To perform a statistical comparison of the bacterial microbiome across species, a rarefaction process was applied to standardize the number of sequences across all samples. Based on this normalized matrix, four alpha diversity metrics were calculated: amplicon sequence variants (ASVs), Shannon index, Pielou's evenness, and Faith's phylogenetic diversity. Differences between groups were assessed using the nonparametric Kruskal–Wallis test ($p < 0.05$). Subsequently, a post hoc analysis was performed using Dunn's uncorrected test for multiple comparisons between pairs of groups, and the significant metrics were visualized using boxplots in GraphPad Prism software (version 8.0.2).

For beta diversity analysis, four metrics were used to assess differences in microbial composition: (1) Jaccard (Jaccard, 1912), which is based on the presence or absence of taxa, allowing for the evaluation of qualitative similarities between microbial communities; (2) Bray–Curtis (Bray and Curtis, 1957) which considers the relative abundance of taxa, providing a quantitative measure of dissimilarity; and the UniFrac metrics, which incorporate phylogenetic information: (3) unweighted UniFrac, which considers only the presence or absence of lineages, and weighted UniFrac (Lozupone and Knight, 2005), which also includes their relative abundance. These metrics generate dissimilarity values ranging from

0 to 1, where values close to 0 indicate similar communities and values close to 1 reflect highly different communities. Differences in the structure of microbial communities between species were analyzed using a permutational multivariate analysis of variance (PERMANOVA, $p < 0.05$). Significant beta diversity metrics were represented using principal coordinate analysis (PCoA), which allows for the visualization of sample clustering or separation based on their composition; these plots were generated in Emperor (Vázquez-Baeza et al., 2013).

Additionally, a similarity percentage analysis (SIMPER) (Khomich et al., 2021) was performed to identify the taxa that contribute most to the dissimilarity among cows, goats, and sheep at the phylum, family, and species levels, using the PAST software (version 5.1, University of Oslo, Norway). Subsequently, Mann–Whitney tests ($p < 0.05$) were applied to taxa whose contribution exceeded 0.1%. Finally, bacterial taxa with significant differences were represented using heatmaps generated in BioRender.

3. RESULTS

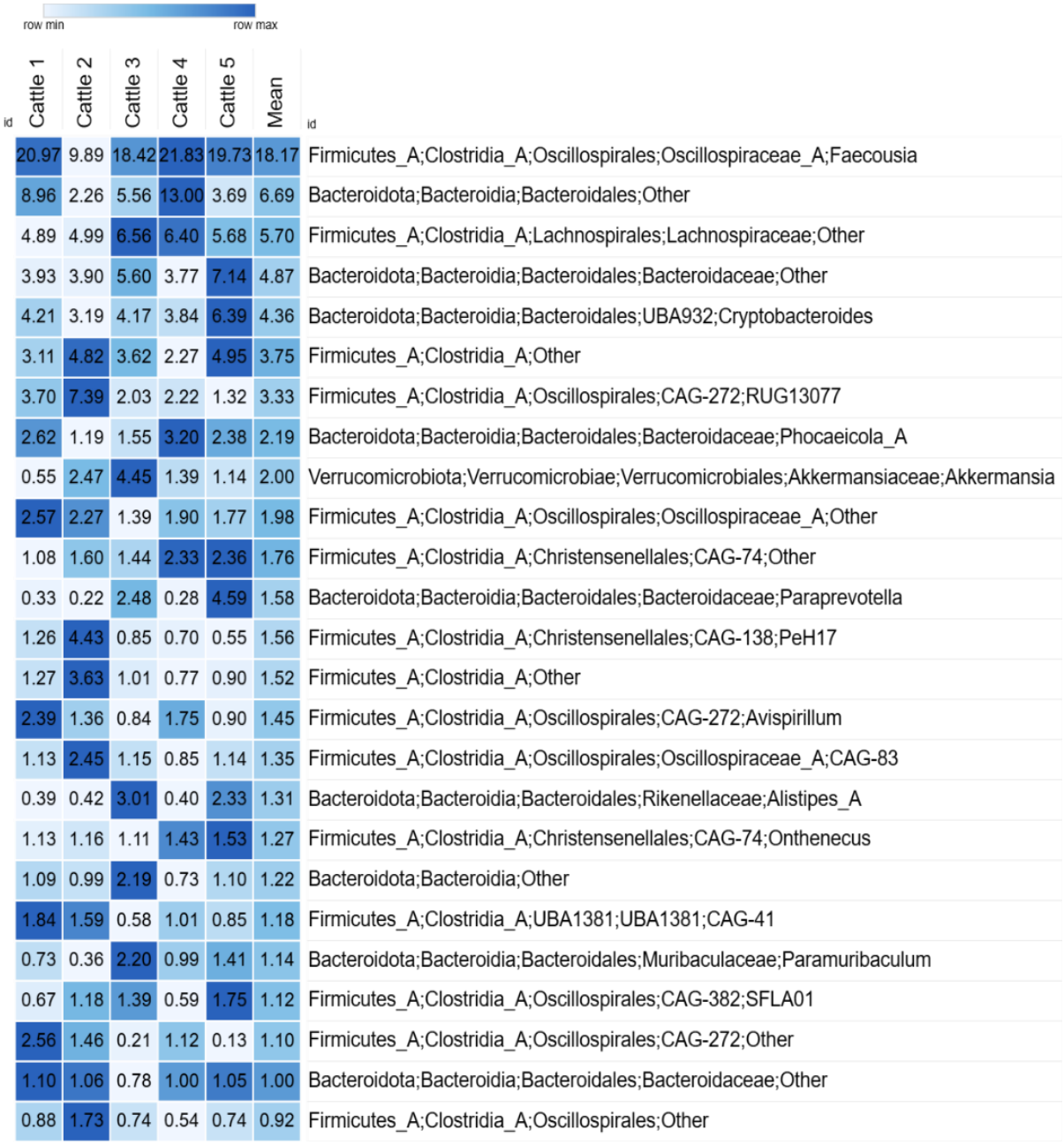
The average number of reads obtained was 197,532.0 for cattle, 195,840.2 for goats, and 206,016.0 for Barbary sheep. The average number of non-chimeric sequences was 79,583.8 (40.45%) in cattle, 102,406.6 (50.82%) in goats, and 88,622.4 (43.04%) in Aoudad sheep. Good's coverage values were 0.999 for each sample, indicating that the sequencing depth was sufficient to capture most of the bacterial diversity. The total number of ASVs was 19,218 (8,477 corresponded to cattle, 5,929 to goats, and 6,040 to Aoudad sheep). Taxonomic richness at the genus level varied among the analyzed species. Cattle had 431 identified genera,followed by goats with 405, and finally Barbary sheep with 356 genera. These results demonstrate greater diversity in cattle (Table 1).

Table 1. Bacterial taxonomic diversity at the phylum, class, order, family, genus, and species levels in cattle, goats, and Barbary sheep.

Species	Phylm	Class	Order	Family	Genus	Species
Cattle	24	44	101	179	431	584
Goat	23	38	100	179	405	528
Aoudad	22	33	76	145	356	464

In the three species analyzed, the predominant phyla were Firmicutes_A and Bacteroidota. In cattle, Firmicutes_A showed the highest average abundance (63.49%), followed by Bacteroidota (27.53%). In goats, Firmicutes_A was more dominant (69.59%), while Bacteroidota accounted for a smaller proportion (20.87%). In Barbary sheep (aoudad), the pattern was similar, with Firmicutes_A as the most abundant phylum (59.51%) and Bacteroidota in second place (27.40%). At the family level, Oscillospiraceae_A was the most abundant in all three species, with average values of 23.76% in cattle, 23.46% in goats, and 19.16% in Barbary sheep. In cattle, the second most abundant family was

Bacteroidaceae (10.34%), while in goats and Barbary sheep it was Lachnospiraceae, with 22.93% and 12.37%, respectively. At the genus level, Faecousia stood out as the most abundant genus in all three species, with average values of 18.17% in cattle (Figure 2), 17.22% in goats (Figure 3), and 13.39% in Barbary sheep (Figure 4).



Cattle 1	Cattle 2	Cattle 3	Cattle 4	Cattle 5	Mean	id
20.97	9.89	18.42	21.83	19.73	18.17	Firmicutes_A;Clostridia_A;Oscillospirales;Oscillospiraceae_A;Faecousia
8.96	2.26	5.56	13.00	3.69	6.69	Bacteroidota;Bacteroidia;Bacteroidales;Other
4.89	4.99	6.56	6.40	5.68	5.70	Firmicutes_A;Clostridia_A;Lachnospirales;Lachnospiraceae;Other
3.93	3.90	5.60	3.77	7.14	4.87	Bacteroidota;Bacteroidia;Bacteroidales;Bacteroidaceae;Other
4.21	3.19	4.17	3.84	6.39	4.36	Bacteroidota;Bacteroidia;Bacteroidales;UBA932;Cryptobacteroides
3.11	4.82	3.62	2.27	4.95	3.75	Firmicutes_A;Clostridia_A;Other
3.70	7.39	2.03	2.22	1.32	3.33	Firmicutes_A;Clostridia_A;Oscillospirales;CAG-272;RUG13077
2.62	1.19	1.55	3.20	2.38	2.19	Bacteroidota;Bacteroidia;Bacteroidales;Bacteroidaceae;Phocaeicola_A
0.55	2.47	4.45	1.39	1.14	2.00	Verrucomicrobiota;Verrucomicrobiae;Verrucomicrobiales;Akkermansiaceae;Akkermansia
2.57	2.27	1.39	1.90	1.77	1.98	Firmicutes_A;Clostridia_A;Oscillospirales;Oscillospiraceae_A;Other
1.08	1.60	1.44	2.33	2.36	1.76	Firmicutes_A;Clostridia_A;Christensenellales;CAG-74;Other
0.33	0.22	2.48	0.28	4.59	1.58	Bacteroidota;Bacteroidia;Bacteroidales;Bacteroidaceae;Paraprevotella
1.26	4.43	0.85	0.70	0.55	1.56	Firmicutes_A;Clostridia_A;Christensenellales;CAG-138;PeH17
1.27	3.63	1.01	0.77	0.90	1.52	Firmicutes_A;Clostridia_A;Other
2.39	1.36	0.84	1.75	0.90	1.45	Firmicutes_A;Clostridia_A;Oscillospirales;CAG-272;Avispirillum
1.13	2.45	1.15	0.85	1.14	1.35	Firmicutes_A;Clostridia_A;Oscillospirales;Oscillospiraceae_A;CAG-83
0.39	0.42	3.01	0.40	2.33	1.31	Bacteroidota;Bacteroidia;Bacteroidales;Rikenellaceae;Alistipes_A
1.13	1.16	1.11	1.43	1.53	1.27	Firmicutes_A;Clostridia_A;Christensenellales;CAG-74;Onthenecus
1.09	0.99	2.19	0.73	1.10	1.22	Bacteroidota;Bacteroidia;Other
1.84	1.59	0.58	1.01	0.85	1.18	Firmicutes_A;Clostridia_A;UBA1381;UBA1381;CAG-41
0.73	0.36	2.20	0.99	1.41	1.14	Bacteroidota;Bacteroidia;Bacteroidales;Muribaculaceae;Paramuribaculum
0.67	1.18	1.39	0.59	1.75	1.12	Firmicutes_A;Clostridia_A;Oscillospirales;CAG-382;SFLA01
2.56	1.46	0.21	1.12	0.13	1.10	Firmicutes_A;Clostridia_A;Oscillospirales;CAG-272;Other
1.10	1.06	0.78	1.00	1.05	1.00	Bacteroidota;Bacteroidia;Bacteroidales;Bacteroidaceae;Other
0.88	1.73	0.74	0.54	0.74	0.92	Firmicutes_A;Clostridia_A;Oscillospirales;Other

Figure 2. Heat maps of the most abundant microbial community at the genus level in fecal samples from cattle. The top 25 genera were selected based on their relative abundance.

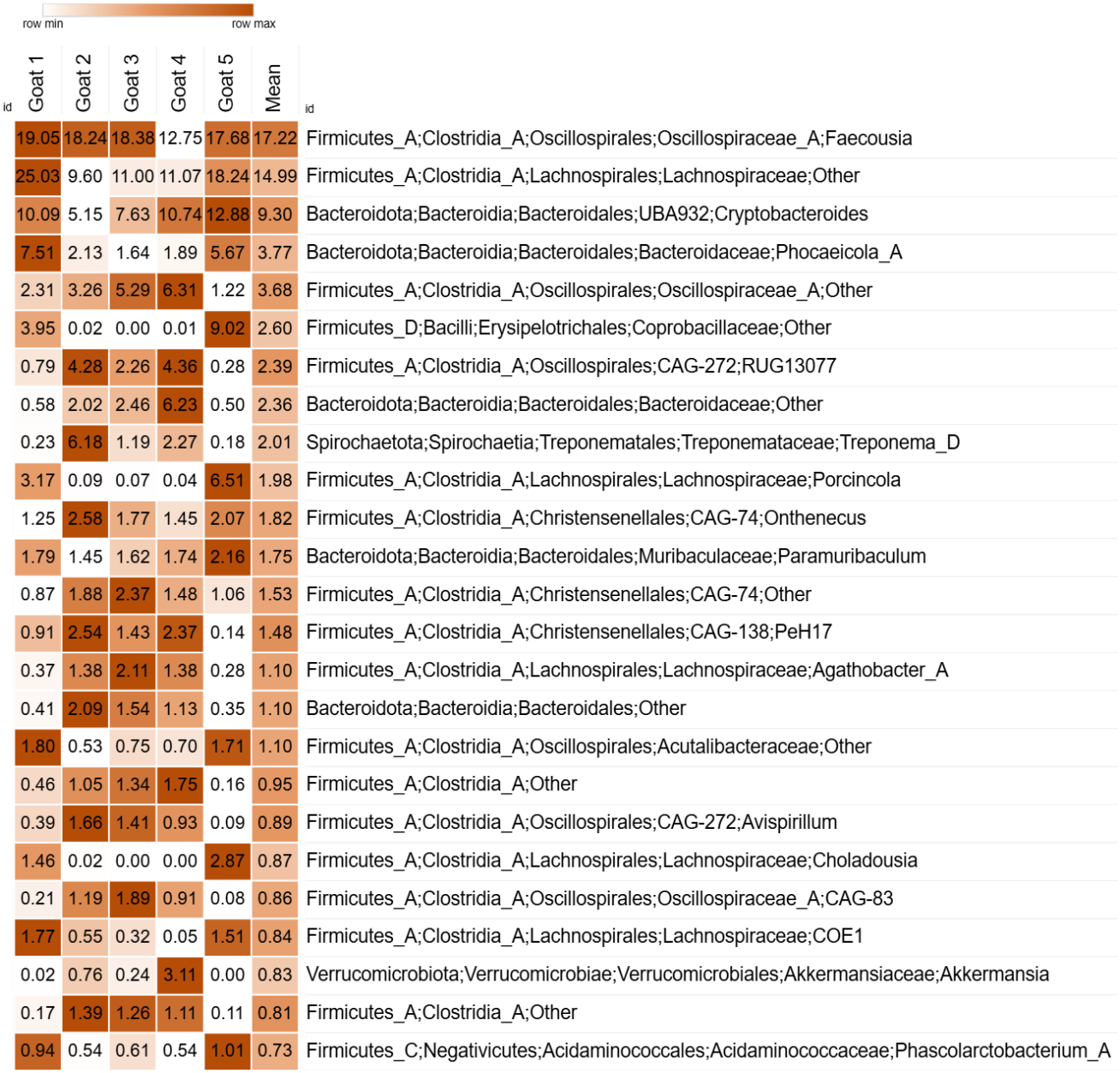


Figure 3. Heat maps of the most abundant microbial community at the genus level in fecal samples from goats. The top 25 genera were selected based on their relative abundance.

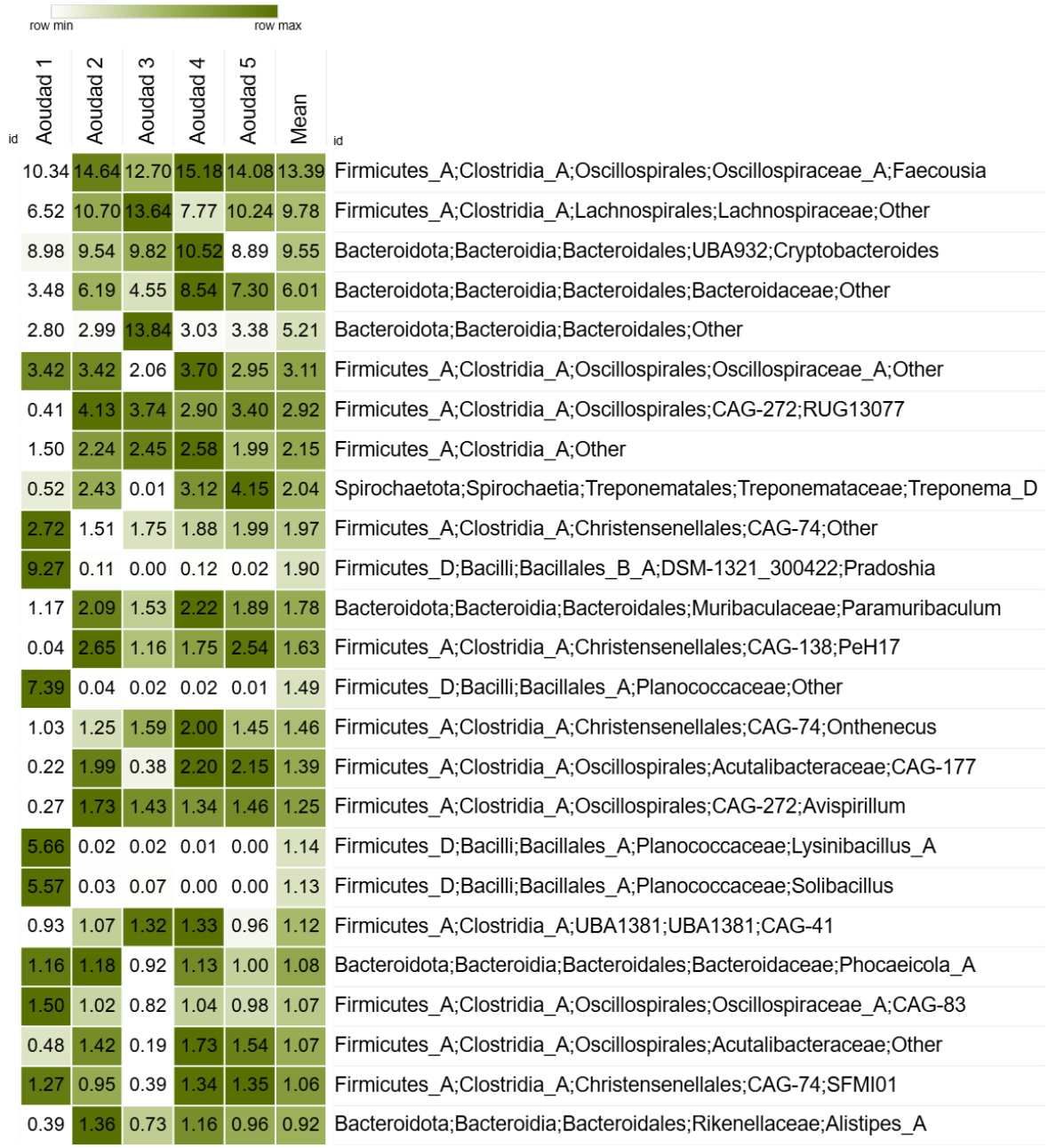


Figure 4. Heat maps of the most abundant microbial community at the genus level in fecal samples from Barbary sheep. The top 25 genera were selected based on their relative abundance.

No significant differences were observed in three of the four alpha diversity metrics evaluated among the analyzed species: number of ASVs (H = 5.040, p = 0.0746; cattle = 2,425, goats = 1,565, Barbary sheep = 1,888), Shannon index (H = 0.320, p = 0.8710; cattle = 9.256, goats = 8.103, Barbary sheep = 8.800) and Pielou's evenness (H = 0.560,p = 0.7825; cattle = 0.8254, goats = 0.7714, Barbary sheep = 0.8091). In contrast, Faith's phylogenetic diversity showed significant

differences among the species (H = 6.080, p = 0.0396), with cattle exhibiting the highest values (mean = 150.8), compared to goats (mean = 98.64) and Barbary sheep (mean = 113.7) (Figure 5).

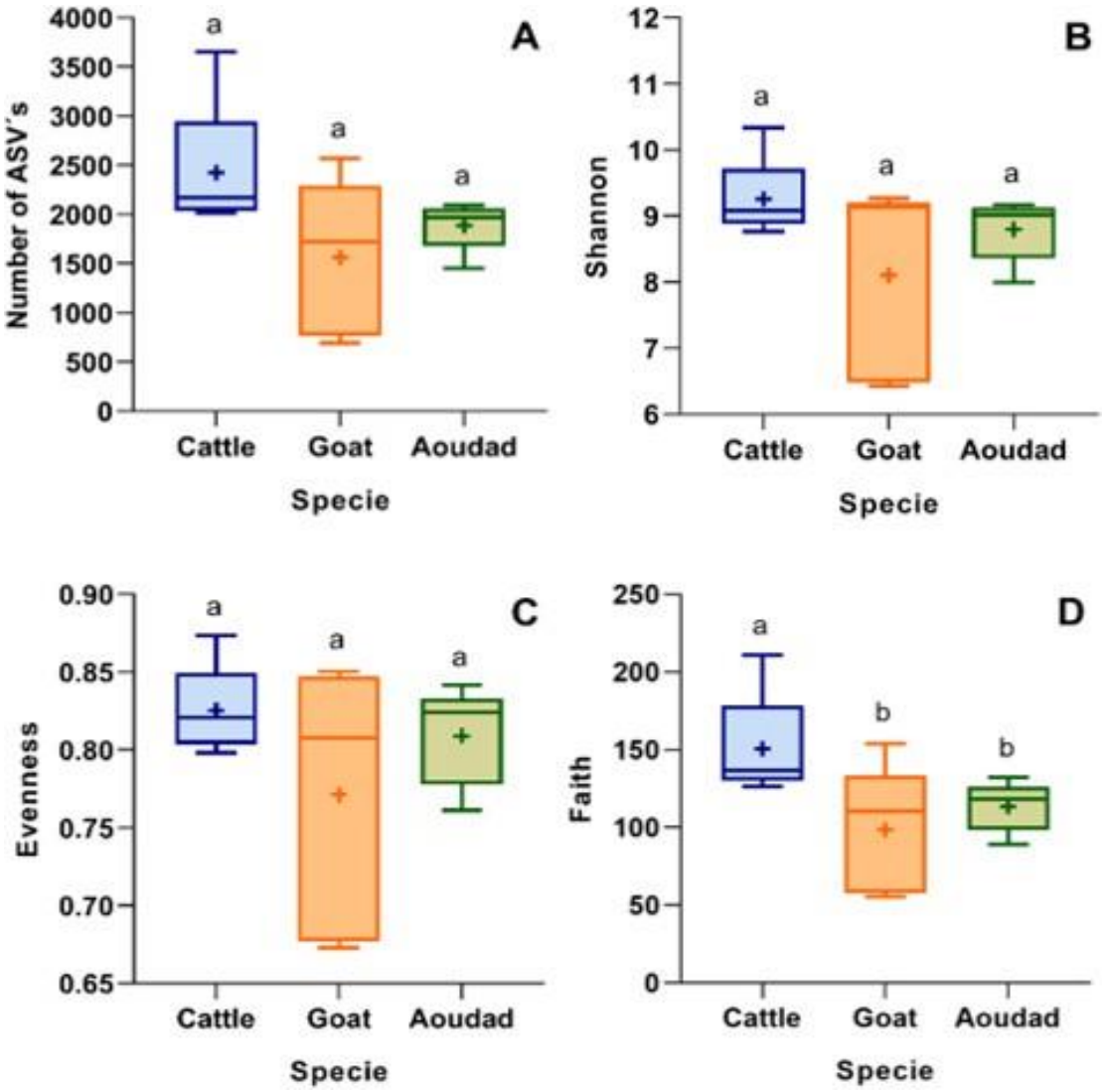


Figure 5. Different lowercase letters indicate significant differences between groups ($p < 0.05$; Kruskal–Wallis test followed by Dunn's post hoc test, without correction).

Regarding beta diversity, all four indices showed significant differences among the analyzed species: the Jaccard index (PERMANOVA pseudo-F = 2.246738, p = 0.001), Bray–Curtis (PERMANOVA pseudo-F = 3.816899, p = 0.001), unweighted UniFrac(PERMANOVA pseudo-F = 1.759157, p = 0.001), and weighted UniFrac (PERMANOVA pseudo-F = 3.001395, p = 0.002). The principal coordinate analysis (PCoA) plots show a separation among the three species ( ) analyzed for the four beta diversity indices, although with some overlap between certain groups (Figure 6).

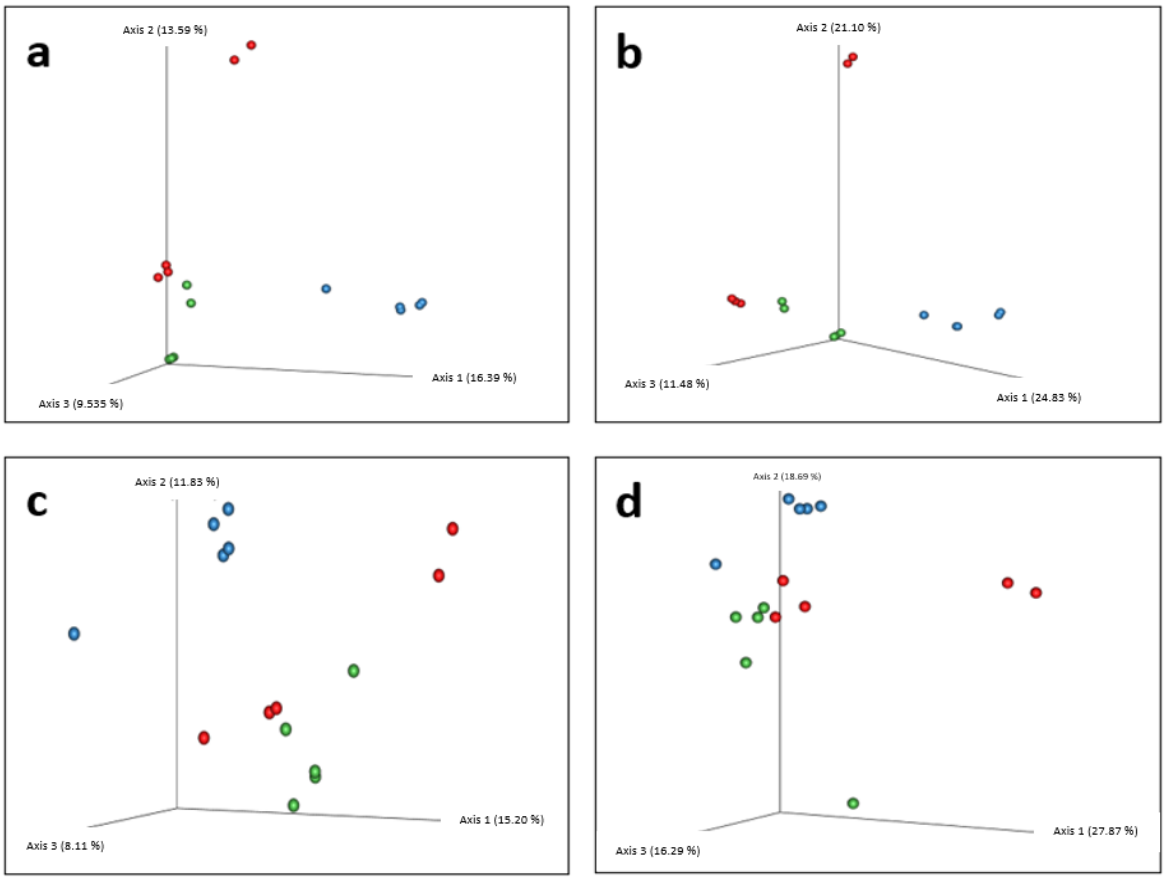


Figure 6. Principal coordinate analysis (PCoA) plots based on the Jaccard (a), Bray–Curtis (b), unweighted UniFrac (c), and weighted UniFrac (d) distance metrics of the fecal bacterial microbiota in cattle (blue dots), goats (red dots), and arruis (green dots).

According to the SIMPER analysis, the overall dissimilarity at the phylum level among the analyzed species was 22.3%. At this level, two taxa with significant differences ($p \leq 0.05$) were identified, corresponding to Other_1 (DC = 0.7473%, H = 6.9, p = 0.03054) and Firmicutes_B_370539 (DC = 0.5553%, H = 6.66, p = 0.03579). At the family level, the overall dissimilarity was 35.21%, with a total of 24 taxa showing significant differences (Figure 7). At the genus level, the overall dissimilarity increased to 42.78%, making it thelevel with the greatest differentiation. In this case, 86 taxa with significant differences were identified, of which the top 25 are presented in Figure 8, highlighting genera such as Faecousia and Cryptobacteroides.

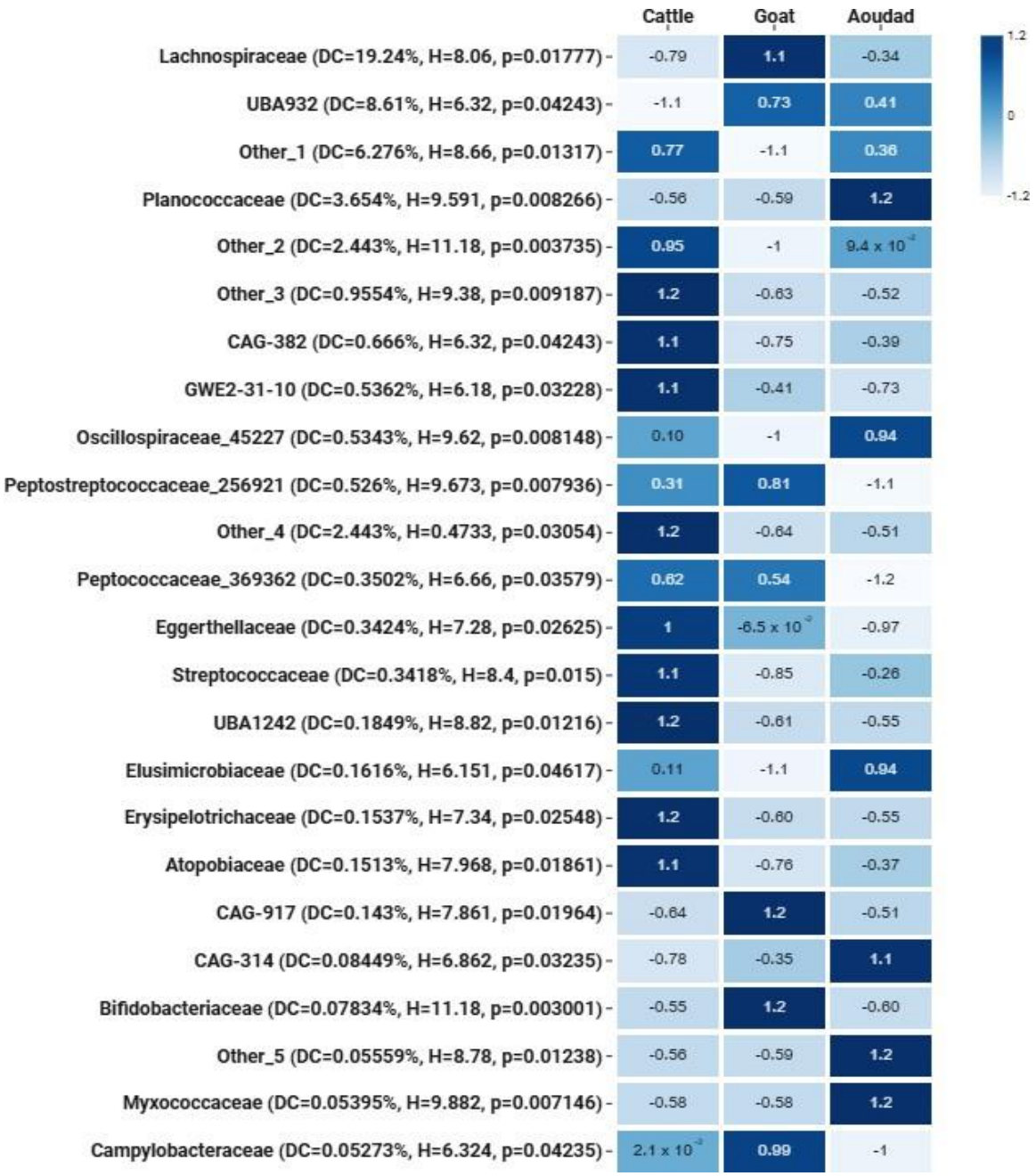


Figure 7. Family-level heat map of the fecal bacterial microbiota in cattle, goats, and arruis, highlighting the enriched taxa with significant differences between species. DC = percentage contribution to dissimilarity, H = Kruskal–Wallis test.

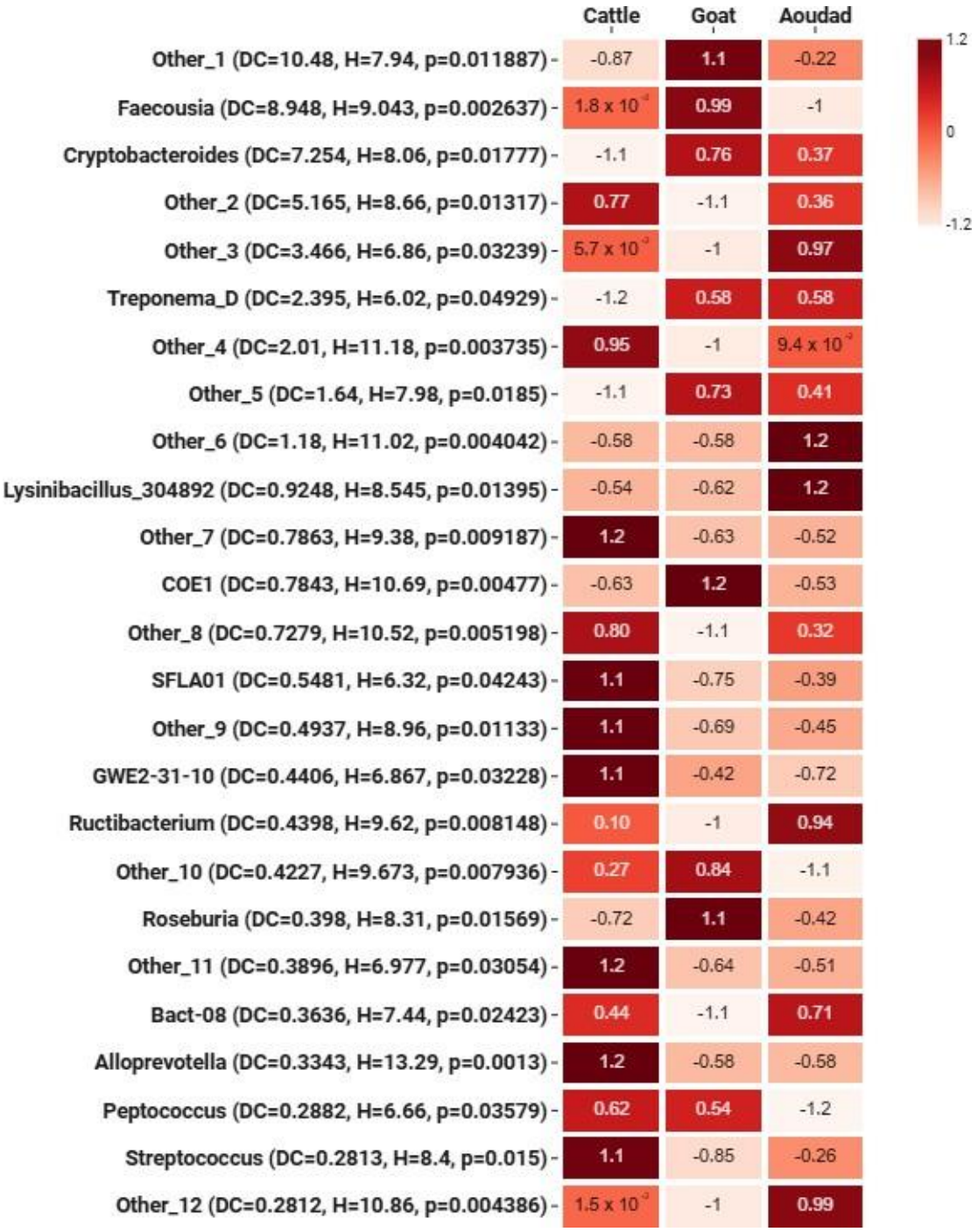


Figure 8. Family-level heatmap of fecal bacterial microbiota in cattle, goats, and arruis, highlighting the top 25 enriched taxa with significant differences between species. DC = percentage contribution to dissimilarity, H = Kruskal–Wallis test.

4. DISCUSSION

4.1 General summary of key findings

The present study provides novel evidence on the structure and diversity of the fecal microbiome in wild and domestic ruminants under sympatric conditions within an arid ecosystem. In general terms, the results revealed that, although there is a marked similarity in taxonomic composition at the phylum level—dominated by Firmicutes and Bacteroidota—significant differences were also observed in community structure (beta diversity) and phylogenetic diversity between species. These findings suggest a dual pattern of microbial convergence and divergence, likely determined by the interaction between shared ecological factors and intrinsic host characteristics.

This pattern is consistent with recent studies that have documented that the gut microbiome in ruminants simultaneously reflects the influence of the environment and the host's evolutionary history (Reese et al., 2021). In this context, our results support the hypothesis that sympatry may favor partial homogenization of the microbiome, without completely eliminating host specificity.

4.2 Structure of the gut microbiome in arid ecosystems

The dominance of Firmicutes and Bacteroidota observed in the three species is consistent with what has been widely reported in domestic and wild ruminants (Henderson et al., 2015; Auffret et al., 2017). These phyla perform key functions in the degradation of structural polysaccharides and in the production of volatile fatty acids, which are essential for host nutrition.

In arid ecosystems, where forage availability and quality are limited, the efficiency of the gut microbiome plays a critical role in animal adaptation (de Jonge et al., 2022). The high proportion of Firmicutes observed, particularly in goats, could be associated with a greater capacity for energy utilization, which has been linked to nutrient-poor, high-fiber diets (Chen et al., 2022).

Likewise, the stability in composition at the phylum level suggests that, despite differences between species, there are common environmental pressures that shape the basic structure of the microbiome in this type of extreme ecosystem.

4.3 Microbial convergence induced by sympatry

One of the most relevant findings of the study is the evidence of partial similarity in microbial composition between species, suggesting a process of convergence associated with the shared use of habitat and forage resources. This phenomenon has been documented in other systems where ecological proximity favors the transfer of microorganisms between species (Pascoe et al., 2017).

Microbial convergence can be explained by multiple mechanisms, including the ingestion of microorganisms present in the environment, indirect coprophagy, and contact with contaminated surfaces (Song et al., 2013). In extensive systems, where cattle, goats, and wildlife share grazing areas, these processes can intensify, generating partially overlapping microbial communities.

From an ecological perspective, this pattern suggests that the microbiome can act as a dynamic component of the ecological niche, responding rapidly to changes in diet and the environment.

4.4 Microbial divergence and host specificity

Despite the observed convergence, beta diversity analyses revealed significant differences between species, indicating that host identity remains a determining factor in microbiome structure. This finding aligns with recent studies highlighting the strong phylogenetic signal of the gut microbiome in mammals (Reese et al., 2021).

Differences in digestive physiology, feeding behavior, and metabolism may explain this divergence. For example, cattle have a larger ruminal volume and different feeding patterns than goats, which may influence microbial composition (Weimer, 2020). Likewise, the Barbary sheep, as a wild species adapted to extreme conditions, may harbor specialized microbial communities that reflect its evolutionary history.

This balance between convergence and divergence highlights the complexity of the factors shaping the gut microbiome.

4.5 Alpha and phylogenetic diversity: ecological implications

The absence of significant differences in indices such as Shannon and Pielou suggests that microbial richness and evenness are relatively similar across species. However, the greater phylogenetic diversity observed in cattle indicates a broader evolutionary range of microbial communities in this species.

This result could be related to greater dietary diversity or to management practices that increase exposure to different microorganisms (Reese et al., 2021). Recent studies have indicated that phylogenetic diversity may be associated with greater functional stability of the microbiome (Lozupone et al., 2012), which could have implications for resilience to environmental changes.

The identification of dominant genera such as *Faecousia* and *Cryptobacteroides* suggests an important role in the fermentation of complex compounds and the production of energy metabolites. Although the specific function of some of these taxa has not yet been fully characterized, recent studies have shown that members of families such as Oscillospiraceae and Lachnospiraceae are associated with the production of butyrate and other volatile fatty acids (Yang et al., 2021).

These functions are particularly relevant in arid environments, where metabolic efficiency is key to survival.

4.6 The wildlife–livestock interface and the One Health perspective

The coexistence of wild and domestic ruminants has significant implications from a One Health perspective. The potential transfer of microorganisms, including pathogens and antimicrobial resistance genes, has been widely documented in

systems where there is close interaction between wildlife and livestock (Allen et al., 20; Vittecoq et al., 2016). Although this study did not directly assess the resistome, the observed microbial similarity suggests that these processes may be occurring in the study area. This highlights the need to integrate multidisciplinary approaches that consider animal, human, and environmental health.

The Barbary sheep is an introduced species with potential ecological impacts on arid ecosystems. The characterization of its microbiome provides relevant information on its adaptive capacity and its interaction with domestic species.The partial similarity in the microbiome suggests that this species can functionally integrate into the ecosystem, which could facilitate its expansion. However, it also poses risks associated with competition for resources and the disruption of ecological processes.

5. CONCLUSION

This study demonstrates that sympatry between wild and domestic ruminants in arid ecosystems generates complex patterns of microbial convergence and divergence. These findings contribute significantly to the understanding of microbiome ecology and have relevant implications for conservation and animal production.

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CAPITULO 5. CONCLUSIONES GENERALES

En conjunto, esta síntesis integrada muestra que el estudio de la genética de poblaciones y de la ecología del microbioma en mamíferos terrestres ha experimentado una transformación profunda en las últimas décadas, impulsada tanto por el desarrollo de nuevas tecnologías de muestreo y secuenciación como por el uso creciente de enfoques de revisión sistemática y análisis cienciométrico. Estas metodologías han permitido integrar grandes volúmenes de información dispersa, identificar patrones generales y evaluar de manera comparativa tendencias metodológicas, geográficas y taxonómicas en la investigación.

Las revisiones sistemáticas aportan la ventaja de sintetizar de forma estructurada la evidencia disponible, reduciendo el sesgo de selección cuando se aplican criterios claros de inclusión y exclusión. En este caso, permiten comparar directamente la eficacia de métodos invasivos y no invasivos, así como evaluar de manera conjunta variables como la calidad del ADN, la pérdida de alelos o la precisión genotípica. Sin embargo, su principal limitación radica en la heterogeneidad de los estudios incluidos, ya que diferencias en diseño experimental, marcadores utilizados, especies analizadas o métodos estadísticos pueden dificultar la comparación directa y la obtención de conclusiones completamente homogéneas.

Por otro lado, los análisis cienciométricos aportan una visión complementaria al permitir cuantificar la evolución del conocimiento científico, identificar sesgos regionales y taxonómicos, y mapear redes de colaboración y tendencias tecnológicas. Su fortaleza reside en la capacidad de ofrecer una perspectiva macro del desarrollo del campo, revelando dinámicas de crecimiento, cambio de paradigmas metodológicos (por ejemplo, de microsatélites a SNPs) y la expansión de prácticas de ciencia abierta. No obstante, estos enfoques también presentan limitaciones, ya que dependen de bases de datos bibliográficas que pueden estar solamente en el idioma inglés y español.

A nivel metodológico, la evidencia revisada muestra un cambio decisivo hacia el uso de muestreo no invasivo y ADN ambiental, lo que ha ampliado significativamente la cobertura espacial y taxonómica de los estudios, reduciendo al mismo tiempo el impacto sobre el bienestar animal. Sin embargo, estas ventajas se acompañan de limitaciones importantes, como la degradación del material genético, la contaminación y la pérdida de información alélica, lo que exige protocolos estrictos de control de calidad, replicación y estandarización analítica. Asimismo, la transición hacia tecnologías de alta resolución como SNPs y secuenciación de representación reducida ha incrementado la capacidad de detección de variación genética, pero ha introducido desafíos en la comparabilidad entre estudios debido a diferencias en escalas de medición, estructuras de varianza y enfoques analíticos. En este sentido, la interpretación de indicadores como Ho, He y Fis requiere una cuidadosa consideración del sistema de marcadores y de los métodos de corrección utilizados.

Finalmente, la evidencia sobre la interacción entre rumiantes silvestres y domésticos en ecosistemas áridos revela patrones complejos de convergencia y divergencia microbiana, lo que refuerza la importancia de integrar enfoques ecológicos, evolutivos y de conservación. En conjunto, estos resultados destacan que el avance del campo no depende únicamente de la sofisticación tecnológica, sino también de la calidad del diseño experimental, la transparencia en la comunicación científica y la integración de datos a través de metodologías sistemáticas y cienciométricas. Su combinación, junto con la estandarización metodológica y el fortalecimiento de la ciencia abierta, es clave para mejorar la comparabilidad, reproducibilidad e impacto de la investigación en genética de poblaciones y ecología microbiana aplicada a la conservación y la producción animal.