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Comparison of DNA quantity and quality from fecal samples of mammals transported in ethanol or lysis buffer

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ABSTRACT

Using fecal microbial community profiles through sequencing approaches helps to unravel the intimate interplay between health, wellness, and diet in wild animals with their environment. Ensuring the proper preservation of fecal samples before processing is crucial to ensure reliable results. In this study, we evaluated the efficiency of two different preservation methods, considering the following criteria: DNA yield, quality and integrity, and microbial community structure based on Oxford Nanopore amplicon sequencing of the V3-V4 region of bacterial 16S rRNA and protozoa 18S rRNA genes. Eighteen matched pairs of mammalian fecal samples were collected and transported in 99.8% ethanol and lysis buffer; processing occurred between 55 and 461 days post-collection. Wilcoxon signed-rank tests were used to analyze quantitative measurements for paired samples. The A260/280 ratio, a measure of nucleic acid purity, was assessed descriptively for each media, and the Bartlett test evaluated dispersion of this ratio. A Fisher test was performed to compare the number of positive reactions for DNA extraction or PCR amplification of the 16S and 18S rRNA genes between both media. The concentration of total DNA and amplicons, as well as the number of reads obtained in sequencing, was significantly higher in the samples preserved with lysis buffer compared to ethanol, with magnitudes up to three times higher. Electrophoretic analysis of total DNA and amplicons further confirmed superior DNA integrity in lysis buffer preserved samples. The A260/280 values obtained using the lysis buffer were of optimal purity (mean: 1.92) and with little dispersion (SD: 0.27); on the other hand, the ethanol samples also presented an excellent average quality (mean: 1.94), but they were dispersed (SD: 1.10). For molecular studies using mammalian feces, the lysis buffer reagent proved to be a reliable solution for their collection, conservation, and storage.

1. Introduction

In the field of life sciences, the analysis of fecal samples can be helpful in answering different types of research questions related to behavior, population ecology, health, well-being, and diet [1,2]. This analysis can be implemented through different approaches, including microscopy, spectroscopy, biochemical and hormone measurements, as well as molecular biology techniques [3–5]. Such studies offer insights into population genetics, molecular epidemiology, the characterization of gastrointestinal parasites and microbiota, and the detection of organic content in the samples [6,7].

One of the advantages of using fecal samples is the relative ease of collection. This method is noninvasive, especially when researchers can directly observe the animal excreting the stool, as seen in the case of Atelidae primates [8], or when the samples exhibit species-specific characteristics, as in Andean bears, tapirs, felines, and certain domestic animals [9]. Additionally, the relatively low cost associated with fecal sample collection sets it apart from more invasive methods, such as blood draws or tissue biopsies, which may require pre-medication or physical constraints [10,11].

Collecting and storing fecal samples properly is essential to ensure the accuracy of biochemical or molecular measurements [12]. However,

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obtaining high-quality and concentrated DNA from fecal matter samples is a complex task due to the presence of polymerase chain reaction (PCR) inhibitors, including urates, bile salts, complex polysaccharides, bilirubin, and byproducts of digested hemoglobin [13]. Furthermore, fecal samples are inherently contaminated with metabolites from the digestive process, and in the case of samples from wild animals, they may also be exposed to environmental contamination, degradation from exposure to solar radiation, humidity, and inadequate temperatures for preservation of macromolecules [12,14,15]. Additionally, under field conditions, it is often challenging to maintain cold chains to preserve samples until they reach the laboratory [16].

To address these challenges, preservation methods for molecular analyses must protect the target DNA from endogenous nucleases and degrading compounds. Some commercial companies have developed media for environmental DNA preservation, such as DNA stabilizer or RNALater, which have shown promise in improving results with these sample types [17,18]. However, these preservation buffers can be relatively expensive, and their availability may be limited, particularly in developing countries.

An alternative and cost-effective approach involves using ethanol and lysis buffer for collecting and transporting fecal samples [12,13]. In this study, we compare the concentration and quality of DNA obtained from samples collected and transported in both media. We then apply these samples to molecular analysis techniques, such as next-generation sequencing (NGS) using Oxford Nanopore Technology (ONT). Through bioinformatics analysis, we assess various molecular readouts, including DNA integrity, the number of reads obtained for each sample in both media, their conservation capacity, and the ability to establish a taxonomic classification of the microorganisms present [19]. We aim to determine the reliability of these fecal sample preservatives and their ability to stabilize and protect genetic material.

2. Materials and methods

2.1. Collection of fecal samples and storage

Eighteen fecal samples were collected from various species of wild and domestic mammals across different departments of Colombia (Table 1). Each sample was divided into two equal parts, with one portion placed in molecular grade ethanol (99.8%) and the other in a lysis buffer. The lysis buffer was prepared with the following components: 0.1 M Tris-HCl, 0.1 M EDTA, 0.01 M NaCl, and 0.5% SDS at pH 8 [13]. Both sets of samples were transported at room temperature to the laboratory and subsequently stored at −20 °C until extraction. In most cases, the samples were frozen between eight and 20 days after collection. The time (days) elapsed from the collection of each sample to the nucleic acid extraction process is provided in Table 1 (Storage duration).

2.2. DNA extraction and 16S/18S rRNA library preparation

The commercial Kit DNeasy PowerSoil Pro® Kit was used for DNA extraction, according to the manufacturer's instructions with minor modifications (addition of 5 min of stirring). The extracted DNA was diluted in 50 µL of kit resuspension solution. To assess the concentration and quality of extracted DNA, we utilized a Colibri system, (Titertek Berthold). High-quality genomic DNA typically exhibits an OD260/OD280 ratio within the range of 1.8 and 2.0. Values between 1.6 and 1.8 are considered acceptable, while any measurement below 1.6 indicates potential contamination. To evaluate DNA integrity 2 µL of extracted DNA was visualized on a 1% agarose gel (*w/v*) containing GelRed (Biotium®). Electrophoresis was conducted in 0.5× TBE buffer at 100 V for 30 min, using a 1 kb DNA ladder as a molecular weight marker (Thermo Scientific).

For molecular characterization studies, the viability of genomic DNA was determined by amplifying the V3-V4 hypervariable region of the 16S rRNA gene with locus-specific forward primer 341F (5′-CTAYGGGRBGCASCAG-3) and reverse primer 806R (5′-GGACTACNNGGGTATCTAAT-3′) [20]. Additionally, for the 18S rRNA gene we utilized primer G3F1 (5′ –GCCAGCAGCCGCGGTAATTC-3) and primer G3R1 (5′ –ACATTCTTGGCAAATGCTTTCGCAG-3) [21]. Endpoint polymerase chain reaction (PCR) was conducted using the Biorad CFX96 C1000 thermal cycler (Table S1 and Table S2).

To ensure the reliability of the PCR results, each reaction included positive controls using *Pseudomonas* spp. (16S rRNA) and stool from *Tapirus pinchaque* (D001, Table 1) genomic DNA. Negative controls were performed by using PCR-grade autoclaved water in place of template DNA for each PCR.

To visualize the amplicons, we prepared 1.4% agarose gels using a 0.5× TBE buffer solution and stained them with GelRed; Electrophoresis was conducted at 90 V for 40 min, using 100 bp Plus DNA Ladder (Thermo Scientific) as a molecular weight marker. Gels containing both total DNA and amplicons were visualized using a gel scanner (Nippon Genetics, FastGene FAS V model). To determine the concentration of amplicons, we employed a fluorometric method with the Qubit® 3.0 Fluorometer (Thermo Fisher Scientific, United States) using the Qubit™ dsDNA HS Assay Kit following the manufacturer's instructions [22].

2.3. Oxford Nanopore sequencing and bioinformatics

For sequencing of ~450 bp-long amplified regions was conducted using the MinION MK1B sequencing platform, managed by the MinKNOW software [23]. The sequencing library was prepared with the

Table 1
Species and localities of the samples evaluated.

Code	Species	Storage duration (days)	Department	Latitude	Longitude	Elevation
42	Atelidae	67	Guaviare	2.441	−72.689	198
43	*Alouatta seniculus*	57	Caldas	5.189	−75.449	2114
44	*Bos primigenius*	57	Caldas	5.190	−75.449	2218
45	*Equus caballus*	57	Caldas	5.191	−75.451	2118
47	*Equus caballus*	57	Caldas	5.184	−75.449	2314
48	*Alouatta seniculus*	57	Caldas	5.189	−75.449	2110
49	*Alouatta seniculus*	55	Caldas	4.741	−75.595	1809
50	*Bos primigenius*	55	Risaralda	4.743	−75.602	1774
51	*Alouatta seniculus*	55	Quindío	4.755	−75.625	1942
52	*Alouatta seniculus*	55	Quindío	4.715	−75.624	1956
53	*Equus caballus*	55	Quindío	4.713	−75.633	1937
54	*Alouatta seniculus*	55	Quindío	4.710	−75.670	1687
55	*Alouatta seniculus*	55	Quindío	4.708	−75.673	1548
D001	*Tapirus pinchaque*	455	Valle	3.725	−75.951	3743
Eq001	*Equus caballus*	451	Valle	3.717	−75.988	3213
Oso001	*Tremarctos ornatus*	451	Valle	3.713	−75.987	3422
Oso002	*Tremarctos ornatus*	450	Valle	3.701	−75.962	3860
P001	*Puma concolor*	461	Valle	3.705	−75.960	3958

Ligation Sequencing Kit SQK-LSK109 and using the Native Barcoding Expansion Kit 96 (EXP-NBD196). The library pool was sequenced on an R-9.4.1 flow cell and run for 48 h. Raw FAST5 files pass produced were basecalled under high-accuracy mode using the Oxford Nanopore Technologies (ONT) basecaller Guppy v6.2.1. Subsequently, the resulting FASTQ files were employed for taxonomic assignment, a process carried out with Kraken2 [24,25], and the PlusPFP database (3/14/2023). The sequences obtained have been deposited in the NCBI Sequence Read Archive under BioProject number PRJNA1036276, Biosample numbers SAMN38122182 to SAMN38122221, and accession numbers SRR26722958 to SRR26722997.

2.4. Statistical analysis

To compare the concentration of total DNA and amplicons, as well as the reads obtained in the sequencing, we performed a paired samples analysis by evaluating the assumptions of normality and homoscedasticity. The assessment of normality was conducted using the Shapiro-

Table 2
Concentration values from DNA extraction, PCR amplification, and data obtained by Oxford Nanopore Technologies (ONT) sequencing.

Code*	Species	Genomic DNA (ng/μL)	OD260/OD280	Concentration Amplicon 18S rRNA (ng/uL)	Concentration amplicon 16S rRNA (ng/uL)	ONT Reads for 18S rRNA**	ONT Reads for 16S rRNA**
42-BF	Atelidae	54.3	1.9	7.3	NA	38,320	NA
42-OH	Atelidae	1.2	4.9	3.4	NA	28	NA
43-BF	*Alouatta seniculus*	8.9	2.2	9.7	8.9	207,967	291,884
43-OH	*Alouatta seniculus*	9.7	2.4	3.6	9.7	7	74,869
44-BF	*Bos primigenius*	49.6	1.8	8.9	49.6	291,275	177,149
44-OH	*Bos primigenius*	22.4	1.9	7.2	22.4	286,188	159,115
45-BF	*Equus caballus*	50.5	1.8	10.0	50.5	381,698	204,101
45-OH	*Equus caballus*	6.5	2.9	3.4	6.5	177	154,665
47-BF	*Equus caballus*	38.3	1.9	10.9	NA	319,354	NA
47-OH	*Equus caballus*	0.3	0.3	3.6	NA	42	NA
48-BF	*Alouatta seniculus*	17.7	1.8	7.2	NA	227,707	NA
48-OH	*Alouatta seniculus*	12.4	1.8	3.5	NA	291,880	NA
49-BF	*Alouatta seniculus*	206.4	1.9	14.2	NA	156,516	NA
49-OH	*Alouatta seniculus*	10.6	1.5	3.8	NA	69	NA
50-BF	*Bos primigenius*	21.3	1.7	3.2	21.3	18,085	67,914
50-OH	*Bos primigenius*	39.2	1.7	7.1	39.2	21,517	12,468
51-BF	*Alouatta seniculus*	226.8	1.9	13.3	226.8	405,748	1158
51-OH	*Alouatta seniculus*	31.6	1.8	4.7	31.6	211,855	12,080
52-BF	*Alouatta seniculus*	170.1	1.9	12.0	170.1	317,394	294,428
52-OH	*Alouatta seniculus*	15.8	2.6	4.3	15.8	1445	210,534
54-BF	*Alouatta seniculus*	10.3	2.6	NA	NA	NA	NA
54-OH	*Alouatta seniculus*	19.0	2.2	NA	NA	NA	NA
53-BF	*Alouatta seniculus*	23.7	2.0	7.1	NA	44,531	NA
53-OH	*Alouatta seniculus*	6.9	3.5	3.9	NA	24	NA
55-BF	*Alouatta seniculus*	22.5	2.3	6.8	22.5	4529	347,702
55-OH	*Alouatta seniculus*	17.6	1.9	4.5	17.6	31,723	35,986
D1-BF	*Tapirus pinchaque*	23.4	1.6	6.4	NA	60,229	NA
D1-OH	*Tapirus pinchaque*	0.6	0.3	3.8	NA	77	NA
Eq1-BF	*Equus caballus*	42.0	1.7	5.6	42.0	77,208	145,101
Eq1-OH	*Equus caballus*	3.3	1.3	5.4	3.3	265	173,875
Os1-BF	*Tremarctos ornatus*	12.6	2.1	5.9	12.6	50,685	20,567
Os1-OH	*Tremarctos ornatus*	2.1	1.6	7.8	2.1	80	44,382
Os2-BF	*Tremarctos ornatus*	15.7	2.3	NA	NA	NA	NA
Os2-OH	*Tremarctos ornatus*	0.2	0.5	NA	NA	NA	NA
P1-BF	*Puma concolor*	51.5	1.5	2.0	NA	7710	NA
P1-OH	*Puma concolor*	16.2	1.8	16.1	NA	64,781	NA

* BF = lysis buffer, OH = Ethanol.
** NA: Measurement not recorded because the sample didn't amplify.

Wilk test for datasets with <30 data points, while homoscedasticity was evaluated with the Bartlett test. In cases where the concentration did not conform to a normal distribution in some samples ($p < 0.05$) or the homoscedasticity assumption was not met ($p < 0.05$), we conducted comparisons using the non-parametric Wilcoxon test for paired samples, with an alpha level of 0.05. The comparison of the 260/280 ratio was performed descriptively in relation to reference measurements. Data dispersion between ethanol and buffer was assessed using the Bartlett test.

To determine the positivity of the extraction or amplification of the 16 S rRNA and 18 S rRNA genes between the buffer or ethanol media, we conducted a Fisher's exact test. This test compared the number of samples that exhibited a band or the absence of it in the agarose electrophoresis. All statistical analysis were carried out in the Rcmdr 2.8–0 package with R 4.1.2 software [26].

3. Results

The concentration of total DNA and amplicons (18S rRNA and 16S rRNA), as well as 18S rRNA reads extracted from the samples transported in the lysis buffer were significantly higher than those obtained from the samples transported in ethanol ($p = 0.00067$, $p = 0.039$, p = 0.039, and $p = 0.011$, respectively) (Table 2). In the case of 16S rRNA readings, although no significant differences were observed ($p = 0.098$), it's worth noting that the median value was approximately two-fold higher when using the lysis buffer compared to ethanol (Fig. 1).

The A260/280 ratio values for the samples collected in buffer had a mean of 1.92 with a standard deviation of 0.27, which places it between the values of optimal purity for DNA and with negligible dispersion. In contrast, although the samples transported in ethanol exhibited a mean value of 1.94, the deviation was 1.10, reflecting nearly four times greater dispersion compared to the lysis buffer; Only four samples (44-OH, 51-OH, 55-OH, and P1-OH) showed optimal quality ranges (Fig. 1, 260/280). Thus, the variances for both samples were different (Bartlett's K-squared = 25, $p = 5.439E-07$).

The agarose gels used to visualize the total DNA revealed the presence of high molecular weight bands, located above 10,000 bp, which corresponds to the maximum size of the molecular weight marker utilized. While some samples, such as 44BF, 45BF, and 49BF, among others, exhibited a characteristic "sweep" pattern indicative of degraded DNA, 15 out of the 18 samples collected in lysis buffer displayed a complete high-weight band. In contrast, such a band was only visible in four out of the 18 samples collected in ethanol (p-value Fisher's Exact Test = 0.00061) (Fig. 2).

The PCR of the V3-V4 subunit of the 16S rRNA gene presented a higher performance, although not significantly, in samples collected in lysis buffer, in which an amplicon of approximately 450 bp was visible in 16 out of the 18 samples, while in ethanol samples, it was observed in only 11 (p-value Fisher's Exact Test: 0.1212) (Fig. 2). Furthermore, it's worth noting that the amplified band of lysis buffer collected samples, such as 43BF, 45BF, 50BF, and 54BF displayed greater intensity compared to their ethanol collected counterparts. This difference in intensity is likely attributed to variations in DNA concentration and integrity between the two sample sets. In the case of the PCR targeting the 18S rRNA gene, a higher performance was also observed in samples collected in lysis buffer. Approximately 16 samples displayed an amplicon of around 450 bp, whereas in samples collected in ethanol, such a band was observed in only six out of the 18 samples (p-value Fisher's Exact Test: 0.0016) (Fig. 2).

The processed raw reads using Kraken2 revealed that between 87% and 93% of the reads can be classified into some domain for both evaluated genes. Notably, the number of reads obtained for the samples preserved in lysis buffer was greater than those preserved in ethanol. When assessing the 16S rRNA gene, a higher percentage of classified reads corresponded to Bacteria in lysis buffer preserved samples. Similarly, in the case of the 18S rRNA gene, higher percentages were observed in the classification of reads for Fungi and Protozoa in the lysis buffer preserved samples. These findings provide strong evidence of greater genetic material preservation in the samples collected and preserved with lysis buffer, indicating its superior preservation capacity compared to ethanol (Fig. 3, Table S3, and S4).

4. Discussion

Molecular studies of biological samples require appropriate techniques to obtain genomic DNA of high integrity and purity. Ensuring the correct preservation of samples for microbiome studies is essential to obtain accurate and reproducible results. Freezing samples at −80 °C is widely acknowledged as the optimal method for preserving nucleic acids and proteins over time, as it effectively halts degradation without

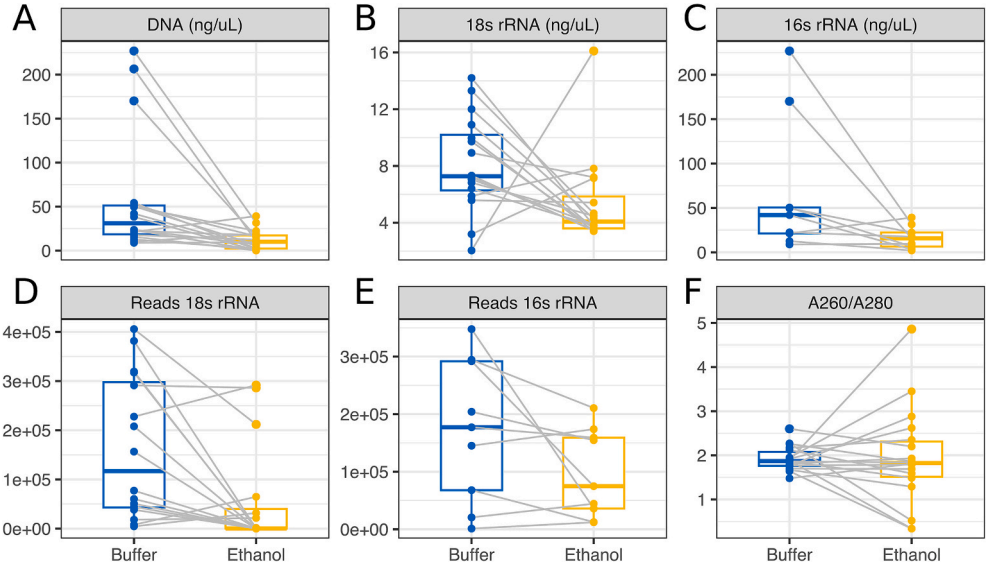

Fig. 1. Boxplot with comparisons between the two transports media (A) genomic DNA concentration (B), 18S rRNA amplicon concentration (C),16S rRNA amplicon concentration, (D) reads ONT for 18S rRNA, (E) reads ONT for 16S rRNA, and (F), DNA quality. Lines connect each pair of samples, buffer – ethanol to each response variable analyzed. It shows the boxplot with samples in interquartile range and outliers. A, B, C, D and E show a depletion in the values to concentration and number of the reads between buffer and ethanol, F shows an increase in the variance.

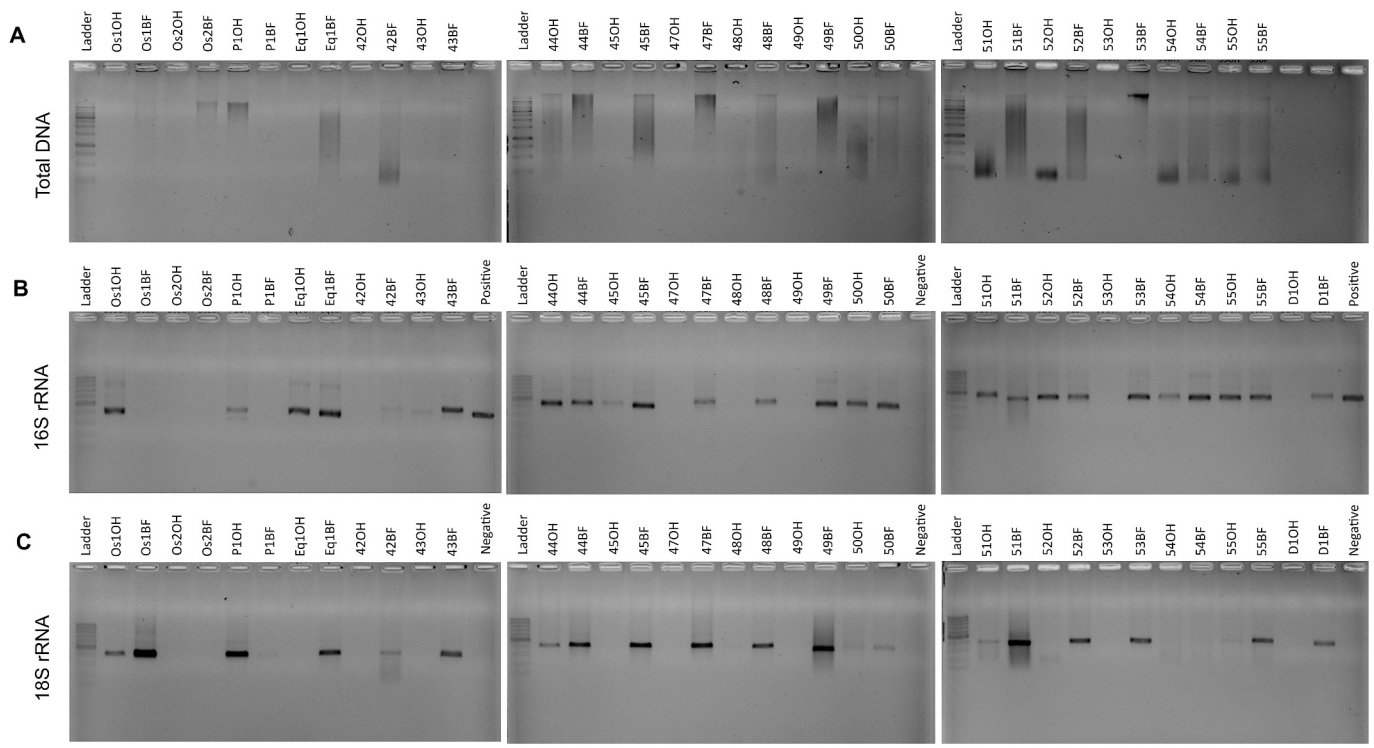

Fig. 2. A. 1% agarose gel of total DNA, stained with GelRed. From left to right; 1: 1 kb molecular weight marker; 2–13: samples. B. 1.4% agarose gel of 16S rRNA gene PCR products, stained with GelRed. From left to right: 1 = 100 bp molecular weight marker, 2–13: samples; Positive: *Pseudomona*; Negative: water negative control. C. 1.4% agarose gel of 18S rRNA gene PCR products, stained with GelRed. From left to right; 1: 100 bp molecular weight marker; 2–13: samples; Positive: positive control *Tapirus pinchaque*; Negative: negative control water. BF = lysis buffer, OH = Ethanol.

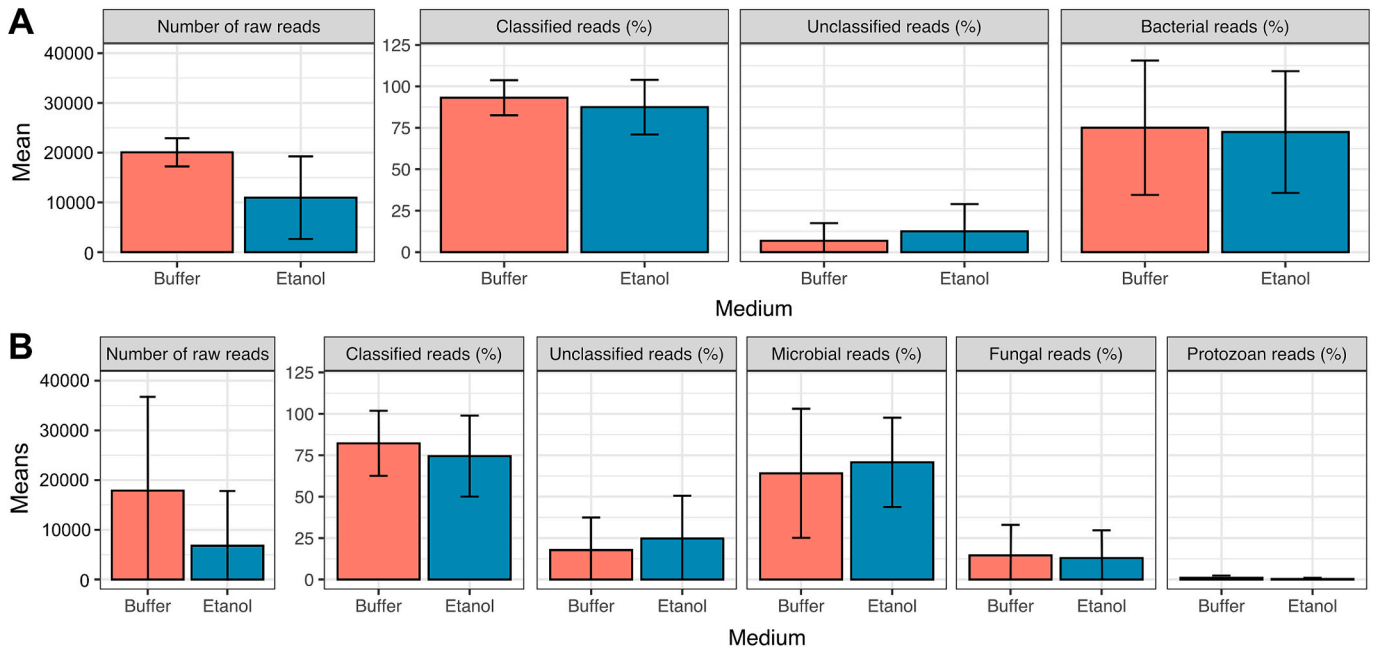

Fig. 3. Data produced and classification by kraken2 for the samples in ethanol and lysis buffer where the 16S rRNA (A) or 18S rRNA (B) genes were amplified.

causing damage to the genome and proteome material of biological specimens [27]. However, in fieldwork, having the necessary equipment for freezing samples is often challenging.

An alternative solution to address this challenge is the preservation of samples in storage solutions. Various preservation media have been discussed in the literature and are available in the biotechnology market. Their use is influenced by factors such as toxicity, resistance to inhibitors, preservation costs, transportation requirements, infectivity of the samples, and laboratory expenses. Considering the non-significant difference in the amount of DNA obtained, the collection of sample in ethanol, as compared to other reagents like 5% potassium dichromate, RNA Later®, Paxgene®, Formalternate® (Ethylene glycol phenyl ether, Phenol, 1,2 -Propanediol), FTA cards, and drying samples with silica or a dehydrator, is often recommended as a pragmatic choice for preserving

fecal samples collected in the field [12,28,29]. It is recognized that the higher the concentration of ethanol, the faster the penetration of cell membranes and the deactivation of nucleases [12].

In our study, the fecal samples transported in the lysis buffer exhibited a higher concentration of total DNA, amplicons, and a greater number of reads obtained during sequencing (Fig. 1, Table 2). Furthermore, the samples collected in the buffer presented superior quality parameters and better gel resolution compared to those transported in ethanol. These qualities improve performance in taxonomic identification. Previous studies have explored various methods of preserving fecal samples to obtain DNA. For instance, research assessing the use of dimethyl sufoxide saline buffer found that this reagent significantly outperformed ethanol, resulting in a 44% increase in amplification success and 17% improvement in genotyping accuracy [30], findings consistent with our study. Additionally, other studies have reported similar results when examining rumen samples to assess microbiota; in these cases, ethanol yielded lower quality outcomes in comparison to two lysis buffers, namely Tris-NaCl-EDTA-SDS and guanidine hydrochlorate [31]. Consistency in results across various studies has led to recommendations favoring the use of buffers for the long-term preservation of DNA extracted from fecal samples [32,33]. Nevertheless, it's essential to recognize that outcomes may vary depending on factors such as the target species, sample type, environmental conditions, or even dietary habits [30]. In our study, the lysis buffer method emerged as the most effective for a diverse array of species, encompassing varying habits and diets, including *Bos primigenius*, *Equus caballus*, *Alouatta seniculus,* and *Tapirus pinchaque*, omnivory in *Tremarctos ornatus*, and carnivory in *Puma concolor* (Table 1). This finding underscores the versatility of the buffer approach for the conservation and preservation of fecal samples from different animal species.

In general, selecting an appropriate method for preserving fecal DNA is critical across all environments, particularly when working with low-quality and degraded samples [30]. However, this consideration becomes more significant in tropical settings where DNA degradation occurs more rapidly. Therefore, optimizing sampling protocols is crucial. The fecal samples used in this study were collected in tropical areas of Colombia, characterized by an average temperature of 28 °C and a relative humidity of 80–90% [34]. Elevated temperatures and increased humidity levels can both negatively impact the preservation of microorganisms in ethanol and lysis buffer. Under high temperatures, ethanol may evaporate, leading to a reduction in its concentration and subsequently diminishing its preserving effectiveness. Similarly, higher relative humidity can also exert a detrimental influence on microorganism preservation within ethanol and lysis buffer. Excessive moisture can condense on sample surfaces, causing dilution of the ethanol or lysis buffer, which may reduce their efficiency [35]. This study highlights the viability of using lysis buffer for molecular studies, including metabarcoding, in such environmental conditions, particularly in tropical regions.

An additional noteworthy observation from our study was the duration between sample collection and long-term storage at freezing temperatures before processing for molecular analysis. Overall, there was no apparent impact of field storage duration on the amount of DNA extracted. Even when samples were stored for extended periods, such as 20 days or up to 461 days at −20 °C, the resulting DNA yields remained relatively consistent (Table 1).

To assess the reliability of two widely used genetic markers for metabarcoding (applicable to bacteria, protozoans, and fungi) in stool samples after various storage durations, we conducted amplification experiments. Our results indicated a higher amplification reliability for the 18S rRNA gene in samples preserved using the lysis buffer as opposed to ethanol. While for the 16S rRNA gene, we observed better performance with the lysis buffer, although it was not significantly different from the performance of samples stored in ethanol.

We utilized next-generation sequencing ONT, a proven and viable alternative for studying intestinal microbiota [36], to analyze the samples. Using the lysis buffer, we obtained twice the number of reads compared to samples preserved with ethanol for both genes under study. However, it's important to note that various factors, such as temperature, storage time, and the concentration of preservation reagents, can influence the results both positively and negatively. For instance, previous research has shown that freezing can increase Firmicutes while benefiting Gram-positive bacteria at room temperature [37]. Furthermore, different ethanol concentrations (e.g., 70% and 95%) can produce varying conservation effects [38]. While some studies recommend 95% ethanol for sample preservation, comparable to cryopreservation's efficacy [38], this study demonstrates superior performance with lysis buffer. An additional benefit of lysis buffer is that it avoids potential transportation restrictions due to ethanol's flammable nature [38].

Considering our findings, we recommend the use of a collection buffer as it requires only a small amount of fecal samples to yield a substantial amount of DNA. It consistently produces high-molecular-weight DNA suitable for various techniques. The buffer is well-suited for field conditions where samples may need to be stored for several weeks at ambient temperatures. These samples can be easily collected into vials pre-filled with lysis buffer. The buffer is also versatile and can be used with various tissues, such as cartilage, blood, striated or smooth muscle, hair follicles, and feathers. Importantly, it does not require refrigeration.

5. Conclusions

The present study assessed and compared the effects of two different stool sample preservation methods. Our experiment showed that the lysis buffer contributed to a higher quality and yield concentrations of DNA and amplification, with higher taxonomic assignment for microbiome composition. This finding offers a viable alternative to rapid freezing for subsequent fecal microbiome analysis, especially in situations where refrigeration and preserving a cold chain during transportation are logistically unfeasible. The collection of fecal samples is vital for investigating prospective associations between the fecal microbiota and the health conditions of wild animals and their environment.

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Institutional review board statement

The animal study protocol was approved by the Ethics Committee of UNIVERSIDAD NACIONAL DE COLOMBIA (UN-17-03-23).

CRediT authorship contribution statement

Néstor Roncancio-Duque: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Resources. **Jeison Eduardo García-Ariza:** Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. **Nelson Rivera-Franco:** Formal analysis, Methodology, Software, Validation, Visualization, Writing – review & editing. **Andrés Mauricio Gonzalez-Ríos:** Data curation, Formal analysis, Methodology, Software, Validation, Writing – review & editing. **Diana López-Alvarez:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2024.100731.

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