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Short Communication

Exploration of environmental DNA (eDNA) signatures of eukaryotes from estuarine sandy beaches in Montevideo, Uruguay

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ABSTRACT. Sandy beaches are dynamic ecosystems supporting diverse life forms and remain less explored in terms of biodiversity compared to other coastal systems. Environmental DNA (eDNA) coupled with sequencing technology has emerged as a non-invasive method for assessing biodiversity, particularly in understudied habitats such as sandy beaches. This study employed eDNA metabarcoding on sediments from four Uruguayan beaches (two urban and two rural) using 18S and COI markers to assess the eukaryotic diversity of eDNA found in sandy beaches. Sediments were collected from the intertidal zone, and DNA was extracted. PCR products were then obtained and subsequently sequenced using high-throughput methods. Taxonomic diversity was analyzed at the family level, categorizing molecular operational taxonomic units (MOTUs) into ecological groups (e.g. algae, fungi, benthos). The 18S marker identified 67% of MOTUs, outperforming the COI marker, particularly for algae, fungi, and microbenthos. Algae had the highest diversity (in terms of the number of MOTUs), followed by microbenthos and fungi, while parasites and zooplankton had fewer MOTUs detected. Beaches showed no clear eDNA diversity differences, although site-specific variations in MOTUs abundance were noted. Some taxa, such as harmful algae and pathogens, have both ecological and health significance. These results demonstrate the utility of eDNA in revealing the diversity of sandy beach DNA and detecting taxa of ecological concern. Expanding spatial and taxonomic coverage, increasing the number of samples, and refining methodologies could further enhance our understanding of biodiversity patterns in these ecosystems.

Keywords: sandy beaches; eDNA; metabarcoding; eukaryotes; Río de la Plata; Uruguay

Sandy beaches are vital ecosystems that host a rich diversity of life forms, making significant contributions to coastal biodiversity. These habitats are dynamic and highly heterogeneous, offering unique niches for organisms ranging from microorganisms to vertebrates (Defeo & McLachlan 2025). Despite their ecological importance, the biodiversity of sandy beaches remains underexplored compared to other marine ecosystems, such as coral reefs and mangroves (Defeo et al. 2021, Lercari 2023). This knowledge gap limits our understanding of the full spectrum of biodiversity these environments support and the ecological roles of less-

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studied groups, such as interstitial meiofauna, microfauna, or fungi (Coull 1999, Danovaro et al. 2008). Comprehensive studies of biodiversity are crucial for understanding the functional roles and evolutionary adaptations of organisms in sandy beach ecosystems. They provide fundamental insights into nutrient cycling, ecosystem stability, and resilience in the face of environmental changes (Schlacher et al. 2008). Additionally, documenting biodiversity *per se* is critical for establishing baseline data, which is indispensable for long-term ecological monitoring and effective conservation strategies. A deeper understanding of sandy beach biodiversity will not only advance ecological science but also inform sustainable management practices for these unique and invaluable ecosystems.

Environmental DNA (eDNA) based techniques have emerged as a promising approach for studying biodiversity in aquatic and terrestrial habitats (Taberlet et al. 2012, Díaz-Ferguson & Moyer 2014, Thomsen & Willerslev 2015). These techniques, based on eDNA, enable the extraction of taxonomic profiles directly from environmental samples, such as water or sediment, without the need to capture or isolate individual organisms. This approach facilitates the detection of a broader diversity of species, including those that are rare, cryptic, or difficult to sample. It allows for long-term monitoring of changes in biological communities (Bohmann et al. 2014, de Faría et al. 2018, Martínez et al. 2021, Weng et al. 2024). In recent years, the application of eDNA has revolutionized marine ecology, providing a non-invasive and high-resolution method for exploring biodiversity in coastal zones (Martínez et al. 2020). Studies have demonstrated its effectiveness in detecting diverse groups of organisms, ranging from microbes to fish, in ecosystems such as coral reefs, deep canyons, and sandy beaches (Guardiola et al. 2015, Stat et al. 2017, Yamamoto et al. 2017, Castro-Cubillos et al. 2022). These advances highlight the potential of eDNA as a key tool for understanding and conserving biodiversity in marine environments.

Estuarine sandy beaches present a unique opportunity to apply eDNA-based techniques and conduct comparative studies. To date, much of the research on beach biodiversity has focused on visible organisms (i.e. macrofauna), often neglecting microscopic eukaryotes that play a crucial role in ecosystem functioning (Pereira-da-Conceição et al. 2020, Horton et al. 2021). Utilizing molecular techniques, such as DNA metabarcoding, allows for the identification of a wider variety of organisms, including taxa from different kingdoms, such as Protista, Fungi, Plantae, and Animalia, thereby offering a more comprehensive understanding of the biological communities present (Macher & Leese 2017, Okamoto et al. 2021). These methods also enhance our ability to assess biodiversity patterns across spatial and temporal scales, which is crucial for understanding ecosystem dynamics and resilience. In particular, recent studies have begun to explore prokaryotic biodiversity in beach sands using molecular techniques, either in environmental health contexts (Whitman et al. 2014) or in relation to contamination (Valerio et al. 2022).

However, despite advances in eDNA metabarcoding, challenges such as limited taxonomic reference databases, biases in amplification methods, and variability in protocols persist, particularly for understudied ecosystems like sandy beaches, where applications remain scarce (Taberlet et al. 2012, Thomsen & Willerslev 2015, Castro et al. 2021a). Standardized methods and expanded databases are urgently needed to enhance biodiversity detection in these complex habitats (Eloe-Fadrosh et al. 2016, Pansu et al. 2023).

The objective of this study is to conduct a preliminary methodological exploration of the use of eDNA signatures metabarcoding in estuarine sandy beaches of Uruguay. By considering urbanized and non-impacted rural sandy beaches, we aim to establish a baseline of eukaryotic DNA diversity in these environments. This methodological approach will not only provide an initial assessment of eukaryotic diversity in these beaches but also lay the groundwork for future monitoring and conservation studies in the region. Unlike many studies in this field, which typically focus on specific faunistic groups, our research aims to evaluate the entirety of eukaryotic eDNA present, encompassing algae, fungi, micro-, meio-, and macro-benthos. Additionally, utilizing the swash zone of sandy beaches' ability to function as a vast filter that retains both aquatic and terrestrial materials, we chose to directly analyze the sediment (sand) DNA to capture the greatest possible diversity. Given the limited availability of samples, this work is conceived as a first step towards generating reference data that can be used to detect changes in coastal biodiversity. The formulation of ecological hypotheses or the application of statistical tests to distinguish among beach characteristics or detect specific patterns is not appropriate at this stage, since the number of samples is too limited and lacks replication. This exploratory approach nonetheless provides valuable baseline information for future studies with more robust

experimental designs. Four beaches were sampled along the Montevideo coast in winter 2023: two urban and two rural beaches (Baldeija 2024). Honda (34°53′58.3″S, 56°07′56.8″W) and Pocitos (34°54′36.2″S, 56°08′35.9″W) beaches are highly urbanized, bordered by stone walls and a coastal promenade (Defeo et al. 2024), and face erosion issues linked to dune modifications since the 1970s. In contrast, the rural beaches, Punta Espinillo (34°47′45.0″S, 56°01′11.7″W) and Pajas Blancas (34°51′28.9″S, 56°22′33.3″W), are located in sparsely populated, rural areas (Fig. 1). The four beaches presented similar morphodynamic aspects, such as grain size and slope (Baldeija 2024), factors important for ecological community structure, characterized as microtidal dissipative to intermediate beaches, with gentle slope and fine to medium grain size.

Three sediment samples were collected from different levels of the swash zone (intertidal) to capture potential variations along the intertidal gradient. Samples were transported frozen (-20°C) to the laboratory and subsequently homogenized to obtain a single composite sample of approximately 50 mL of wet sediment per site. From this, DNA was extracted from 0.25 g of sediment using the Quick-DNA™ Fecal/Soil Kit (Zymo Research) within one month of sample collection, following the manufacturer's instructions. Two metabarcoding markers were used: an ~110 bp fragment of the 18S rRNA gene amplified with the 18S_allshorts primers (Guardiola et al. 2015), and a ~108 bp fragment of the COI gene amplified with the BF2/mICOIintR primers (Elbrecht & Leese 2017). The combination of COI and 18S markers is widely recommended in metabarcoding studies, as they provide complementary taxonomic resolution and minimize amplification biases (Haenel et al. 2017, Castro et al. 2021b). PCRs were performed in 15 µL reactions containing 2X MangoMix (Bioline), 10 µM of each primer, 1.2 µL of BSA (10 mg mL^{-1}), and 2 µL of DNA template. Cycling conditions included an initial denaturation at 95°C for 2 min, followed by 40 cycles of 95°C for 5 s, annealing at 55°C (18S) or 59°C (COI) for 15 s, and a final extension at 72°C for 5 min. Positive and negative controls were included in all PCR runs. Amplification success was verified using 1.5% agarose gel electrophoresis. Each sample was indexed with a specific primer tag for identification during unidirectional (single-end) sequencing on the Ion GeneStudio™ S5 System (Thermo Fisher Scientific - IIBCE Platform). Short and low-quality reads ($Q < 28$) were removed using SEED2 software (Větrovský et al. 2018). Due to budget constraints and the exploratory nature of this study, replicates were not processed individually; instead, they were pooled and sequenced together as a single sample. In R, only sequences containing both complete primers were selected, and the primers were subsequently removed. Sequences from both markers were clustered into molecular operational taxonomic units (MOTUs) (Blaxter et al. 2005) using DNA barcode data with a ≥97% similarity threshold, as recommended by Bonin et al. (2023), with the VSEARCH algorithm (Rognes et al. 2016). A BLAST was performed with the most frequent sequence from each cluster using the BLAST+ package (NCBI) against the BLASTBD version 5 (2024). For each analyzed sequence, 500 cluster hits were obtained to define the MOTUs, ranked by e-value; the hit with the lowest value was then selected for further analysis. Taxonomic information for each hit was added using the taxonomizr 0.10.6 package in R. Taxonomic diversity (i.e. number of MOTUs) was analyzed at the family level, as reference databases for these markers may remain incomplete for the study area, limiting reliable assignments at lower taxonomic levels. The families were further categorized into broad ecological groups based on an evaluation of the taxa's living form, size, and kingdom: Algae, Microbenthos, Fungi, Meiobenthos, Plantae, Macrobenthos, Parasites, Zooplankton, and Terrestrial, which includes other taxa inhabiting those environments (e.g. insects or spiders). These categories provide a framework for understanding their ecological roles and interactions within the ecosystem, aligning with different academic schools focused on each category.

Results of eDNA analysis conducted on the sediments from four beaches in Montevideo revealed some apparent differences in the effectiveness of the 18S and COI markers used for detecting taxonomic diversity. The raw reads obtained were higher for the 18S marker, with an average of 122,919 reads, compared to 40,709 reads for the COI marker, reflecting the greater capacity of 18S for detecting eukaryotes, as demonstrated in other studies applying this marker in marine environments (Stat et al. 2017, Sawaya et al. 2019, Martínez et al. 2020). Sixty-seven percent of the operational taxonomic units (OTUs) at the family level were detected using the 18S gene. At the same time, only 32.9% were identified through COI (Fig. 2). These findings align with the literature, which highlights the broader applicability of 18S for detecting eukaryotic diversity, especially in complex benthic communities, such as those on sandy beaches (Taberlet et al. 2012, Pereira-da-Conceição et al. 2020). The COI marker, typically used for identifying marine animal

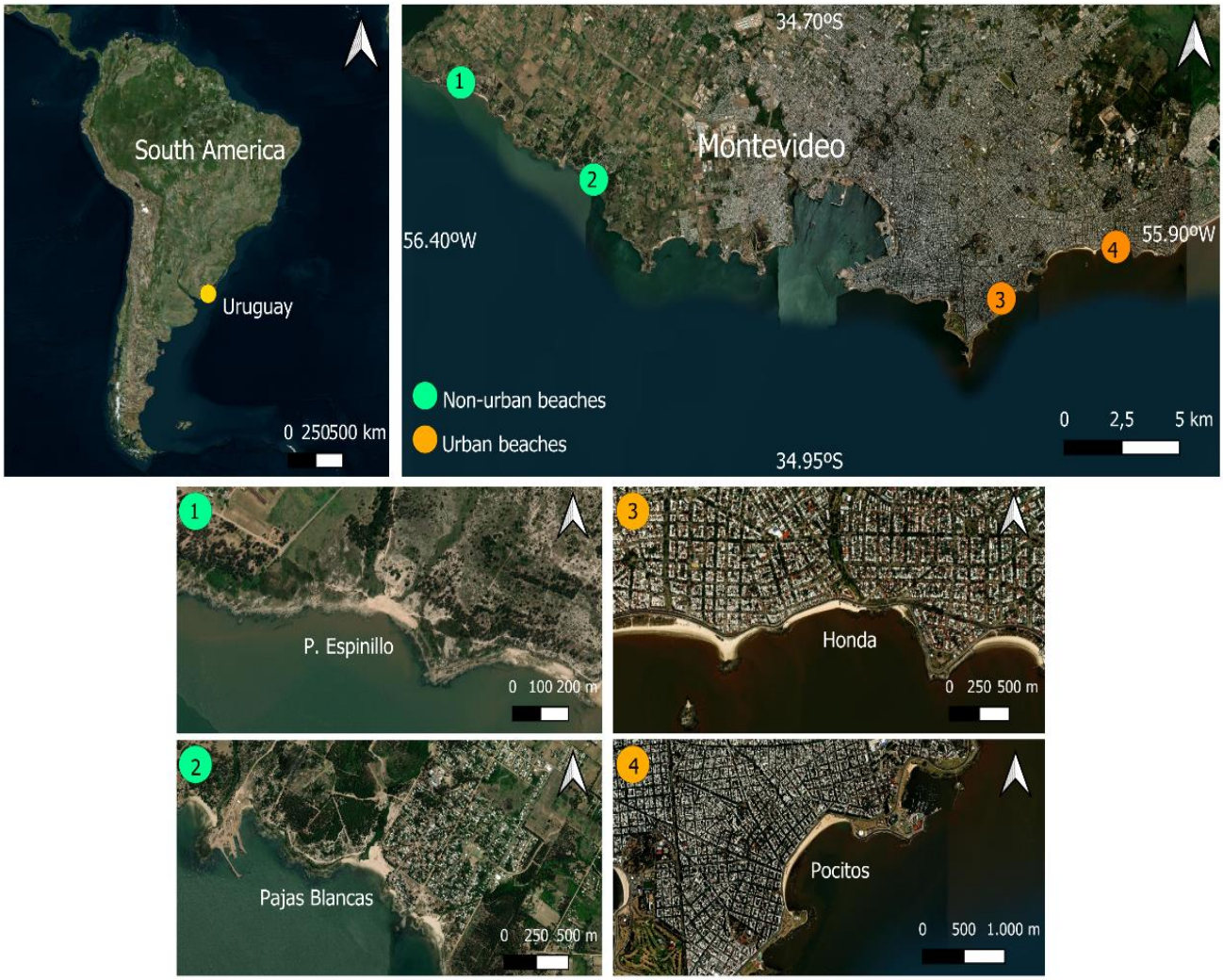

Figure 1. Satellite map showing the location of Montevideo on the Atlantic coast of South America, and a detailed view of its coastline highlighting the four study sites. Urban beaches (orange dots) and rural beaches (green dots) are indicated. The map was created using QGIS software with imagery from Google Earth.

species, proved less effective for groups such as algae and fungi, which are more easily detected using the 18S marker (Bohmann et al. 2014).

Regarding the eDNA composition, algae were the most diverse group, with 53 MOTUs identified by 18S, followed by microbenthos with 47 MOTUs and fungi with 23. This pattern highlights the importance of microbial and benthic groups in coastal ecosystems, where they play essential roles in nutrient cycling and habitat structure (Danovaro et al. 2008, Defeo & McLachlan 2025). The results also emphasize the dominance of microbenthos and meiobenthos, which are crucial in the decomposition of organic matter and trophic interactions within sediments (Coull 1999, Giere 2009, Schratzberger & Ingels 2018). On the other hand, parasites and zooplankton were the least represented, with only 3 and 1 MOTUs, respectively. This result is not surprising, given that our sampling focused on surface sediments rather than water column samples. As noted by Holman et al. (2019), sediments yielded a higher MOTUs richness compared to water samples. However, the choice of sample type (whether water or sediment) should depend on the target organisms of the study. For instance, if the study focuses on plankton, it would make sense to analyze concentrated water samples.

Additionally, this low representation may reflect the inherent challenges of detecting certain taxa using eDNA metabarcoding, particularly organisms with complex life cycles or low DNA concentrations in sedi-

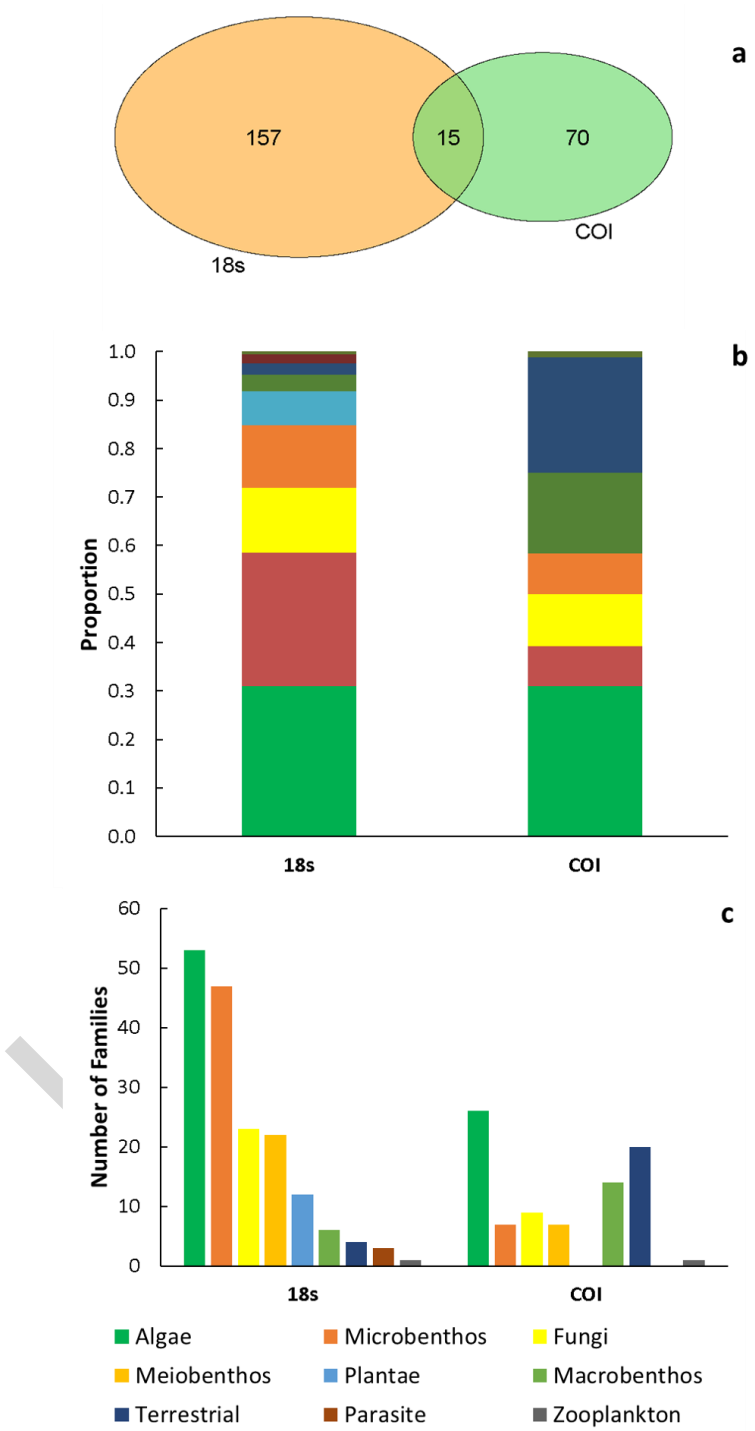

Figure 2. Venn diagram illustrating the number of families identified using the 18S and COI markers, including shared and unique families for a) each marker, b) relative, and c) total number of families identified by the 18S and COI markers for all the sampled sandy beaches along the coast of Montevideo.

ments (Yamamoto et al. 2017). Moreover, eDNA decay further complicates the detection of taxa, as the degradation of eDNA molecules over time can lead to a significant loss of detectable DNA, especially for marine organisms (Holman et al. 2022) which underscores the need for continued development and

Table 1. Taxonomic identity of the five most important families (reads) in each of the defined categories using the 18S marker.

Category	Family	Reads
Algae	Grammatophoraceae	132288
	Rhizosoleniaceae	50906
	Naviculaceae	19629
	Staurosiraceae	12451
	Prorocentraceae	9641
Fungi	Trichosporonaceae	5642
	Malasseziaceae	197
	Filobasidiaceae	180
	Cladosporiaceae	73
	Lobulomycetaceae	48
Macrobenthos	Nereididae	1542
	Enchytraeidae	867
	Serpulidae	281
	Palaemonidae	41
	Balanidae	37
Meiobenthos	Enoplidae	9364
	Xyalidae	1360
	Anoplostomatidae	1123
	Thaumatopsyllidae	661
	Oncholaimidae	520
Microbenthos	Thraustochytriaceae	516
	Nibbleridae	223
	Thaumatomastigidae	121
	Cyphoderiidae	64
	Salpingoecidae	26
Plantae	Poaceae	476
	Cactaceae	40
	Haplomitriaceae	21
	Amaryllidaceae	20
	Oxalidaceae	36
Terrestrial	Chirodiscidae	256
	Archaeidae	160
	Felidae	51
	Psyllidae	50
Parasite	Theileriidae	274
	Eleutheroschizonidae	266
	Aphelidiaceae	47

adjustment of sampling and eDNA extraction protocols and markers to improve the detection of these groups, as recent studies advocate for the expansion of taxonomic reference databases and the use of multiple methodological strategies to achieve a more accurate representation of biodiversity (Castro et al. 2021, Pansu et al. 2023).

Among the most common taxa (Table 1), several are known to dominate marine environments, such as diatoms from the Grammatophoraceae family, or nematodes (meiobenthos) like those in the Enoplidae family (Giere 2009). In addition to nematodes, the relevant families representing the meiobenthos included Karkinorhynchidae (flatworms), Harpacticidae (copepods), Leptocytheridae (ostracods), and Chaetonotidae (gastrotrichs). Similar to the results of Baldeija (2024), flatworms were quite dominant on urban beaches, just as copepods were quite present on non-impacted rural beaches. Also consistent with the bibliography, which characterizes these groups as indicators of impacts such as increased organic matter (Giere 2009, Schratzberger & Ingels 2018). The detected macrobenthic DNA corresponded primarily to families such as Nereididae, Enchytraeidae, Serpulidae, Palaemonidae, and Balanidae. These groups broadly match common components of the benthic fauna previously reported in soft-bottom habitats along the Montevideo coast (Venturini et al. 2004). However, it is noteworthy that no gastropod or bivalve mollusk DNA was detected, despite these being abundant and ecologically relevant taxa in local benthic assemblages. This absence may reflect limitations in DNA preservation, extraction efficiency, or coverage of the reference database, underscoring the need for further exploration and methodological refinement in eDNA surveys of estuarine macrobenthos. Other taxa detected may have significant impacts as potential pathogens, pests, or indicators of environmental problems. For instance, within the Fungi kingdom, the family Trichosporonaceae includes genera such as *Trichosporon*, which are opportunistic fungi capable of causing infections in humans (da Silva et al. 2022). Similarly, the family Malasseziaceae, particularly *Malassezia* species, is associated with common dermatological conditions such as seborrheic dermatitis and dandruff in humans (da Silva et al. 2022). In aquatic environments, dinoflagellates from the family Prorocentraceae can be problematic due to their capacity to form toxic algal blooms, commonly known as red tides, which negatively impact fisheries and human health through biotoxins that accumulate in shellfish (Anderson et al. 2012). In terrestrial ecosystems, the family Psyllidae comprises psyllid insects, notorious agricultural pests that are responsible for transmitting pathogens causing citrus greening disease, a severe threat to citrus crops (Bové 2006). These examples illustrate how eDNA-based techniques can facilitate the identification of problematic species in sandy beaches and surrounding environments, thereby supporting the monitoring and management of biodiversity and ecosystem health (Stat et al. 019).

When comparing the different beaches using the 18S marker, some variations in MOTUs at the family

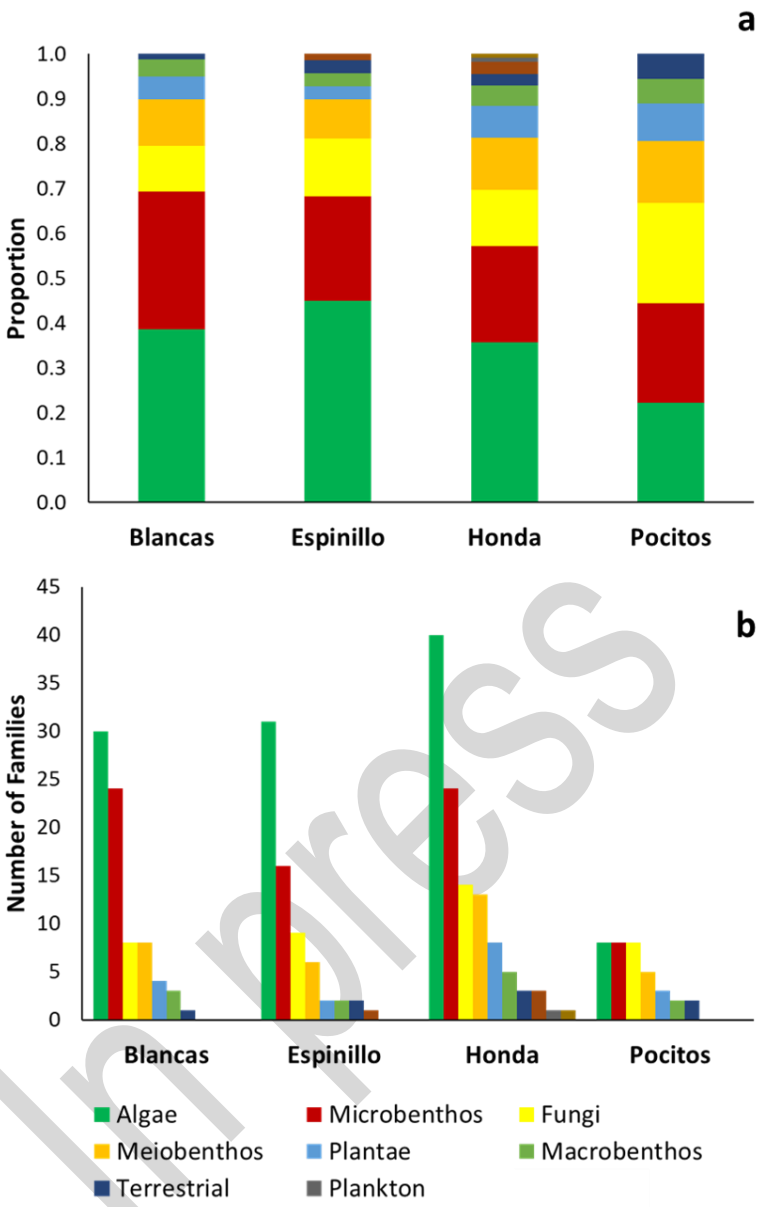

Figure 3. a) Relative and b) total number of families identified by the 18S for rural and urbanized sandy beaches along the coast of Montevideo, Uruguay.

level were observed (Fig. 3a). Honda, an urban beach, showed the highest number of MOTUs in algae, microbenthos, and fungi. Similarly, rural beaches Blancas and Espinillo also exhibited a high number of MOTUs in these categories. In contrast, Pocitos, the other urban beach, showed the lowest number of MOTUs across all categories. Honda and Espinillo had a higher representation of MOTUs in macrobenthos and terrestrial organisms, while parasites were mainly detected in Honda. Zooplankton, although limited, was identified only in Honda and Pocitos (Fig. 2b). Previous studies have highlighted that sandy beaches are highly dynamic ecosystems influenced by both natural and anthropogenic factors, including urbanization and human activity, which can affect biodiversity patterns (Schlacher et al. 2008, Defeo et al. 2009). However, due to our limitation in sample size, the role of urbanization in shaping these patterns remains unclear. In addition, seasonal changes in environmental variables such as sand grain size, salinity, and organic matter content may also play a crucial role in influencing eDNA detection and biodiversity estimates (Pansu et al. 2023, Saenz-Agudelo et al. 2024).

In conclusion, the use of the 18S marker demonstrates its efficacy in identifying a wide diversity of taxonomic groups within intertidal zones. Across beaches, algae, microbenthos, fungi, and meiobenthos ranked highest in MOTU richness. Urban versus rural beach differences were not evident, emphasizing that eDNA-based biodiversity signatures hold promise for holistic biodiversity assessments. However, future studies should incorporate improved spatial and temporal replication, environmental variable integration, and localized reference databases to enhance taxonomic precision (Alfaro-Cordova et al. 2022). Importantly, this method identified DNA from potentially problematic taxa, which pose risks to ecosystems and human health. As a shortcoming of our approach, it is worth noting that the detection of DNA does not confirm the active presence of an organism at a specific site; DNA can also result from transportation, historical deposits, or biological waste (e.g. feces). Longer-term studies, broader spatial analyses, and investigations into DNA degradation dynamics are essential for accurately linking eDNA signatures to living organisms. Pre-triage of samples via size fractionation through sieving can refine the analysis by isolating specific taxonomic groups, such as meiobenthos and macrobenthos, allowing for answering particular ecological questions for each category.

The benefits and limitations of eDNA-based methodologies have been extensively discussed (Beng & Corlett 2020). While they offer high sensitivity for detecting elusive or rare taxa, challenges remain regarding taxonomic resolution, amplification biases across taxonomic groups, contamination risk, and data interpretation, among others. However, their integration with classical taxonomy remains crucial for improving biodiversity assessments. eDNA and traditional surveys often yield complementary data and, when combined, enable a more robust interpretation of community composition and ecological patterns, especially in ecosystems with limited reference data or cryptic species (Qu & Stewart 2019, Tingley et al. 2019, Ji et al. 2020). The standardization of these methodologies, together with comprehensive environmental metadata, will further enhance the application of eDNA in monitoring sandy beach biodiversity and assessing ecosystem health.

Credit the author's contribution

L. Marín: conceptualization, methodology, formal analysis, writing-original draft; G. Botto Núñez: methodology, validation; M. Cosse: funding acquisition, project administration, supervision, review, and editing; B. Baldeija: methodology, results editing; D. Lercari: conceptualization, methodology, formal analysis, writing-original draft, funding acquisition, project administration, supervision, review. All authors have read and approved the final version of the manuscript.

Conflict of interest

All authors have read and accepted the published version of the manuscript. The authors declare no potential conflict of interest in this manuscript.

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