Mammal Review

Mammal Review ISSN 0305-1838

SHORT COMMUNICATION

Assessing the potential of environmental DNA metabarcoding for monitoring Neotropical mammals: a case study in the Amazon and Atlantic Forest, Brazil

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Keywords
Brazil, camera traps, Critically Endangered, eDNA, mammals, river, terrestrial

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Received: 29 August 2019
Accepted: 11 November 2019
Editor: DR

doi: 10.1111/mam.12183

ABSTRACT

The application of environmental DNA (eDNA) metabarcoding as a biomonitoring tool has greatly increased, but studies have focused on temperate aquatic macro-organisms. We apply eDNA metabarcoding to detecting the mammalian community in two high-biodiversity regions of Brazil: the Amazon and Atlantic Forests. We identified Critically Endangered and Endangered mammalian species and found overlap with species identified via camera trapping. We highlight the potential for using eDNA monitoring for mammals in biodiverse regions and identify challenges: we need a better understanding of the ecology of eDNA within variable environments and more appropriate reference sequences for species identification in these anthropogenically impacted biomes.

INTRODUCTION

A quarter of extant mammal species are considered to be threatened (defined as Critically Endangered, Endangered, or Vulnerable) according to the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (IUCN 2019). There is a clear need for more effective and rapid methods for long-term biomonitoring, to be applied in different biomes and over large spatial and temporal scales (Sales et al. 2019a). Environmental DNA (eDNA) metabarcoding (the simultaneous identification via next-generation sequencing of multiple taxa using DNA extracted from environmental samples, e.g. water or soil) is now delivering on its initial potential and is revolutionising how we monitor biodiversity (Deiner et al. 2017). The majority of eDNA

metabarcoding applications have been focused on monitoring fish and macroinvertebrates; mammals have been targeted in only 8% of vertebrate studies (Tsuji et al. 2019). However, with the recent development of universal primers for vertebrates and mammals, there has been a surge in studies tailored to detect and monitor mammalian communities in terrestrial and freshwater environments (e.g. Ushio et al. 2017, Harper et al. 2019, Sales et al. 2019a).

Recent mammal-focused eDNA metabarcoding studies in temperate regions in the northern hemisphere have used well-studied systems with accompanying long-term or historical survey data to test the efficiency of this novel biomonitoring tool (e.g. Harper et al. 2019, Sales et al. 2019a). However, mammal conservation can be more challenging in biodiversity-rich countries, as long-term monitoring systems are still scarce outside of Europe and North America (Proença et al. 2017), and ecological field studies used to plug this gap are often hindered due to difficulties in sampling over wide spatial scales. For effective conservation action, adequate knowledge regarding the biodiversity components present in each area is of paramount importance.

Environmental DNA from lentic and lotic systems has been found to be effective for monitoring not only aquatic and semi-aquatic mammals, but also terrestrial species (Harper et al. 2019, Sales et al. 2019a). We explore the application of eDNA metabarcoding to Neotropical mammals, by verifying its ability to detect aquatic and terrestrial animals from rivers and streams in the highly biodiverse biomes of the Brazilian Amazon and Atlantic Forests. The Amazon is the largest tropical rainforest on earth, encompassing at least 10% of the world's biodiversity. The Atlantic Forest, which is currently only 11% of its original size (Ribeiro et al. 2009), is the second most biodiverse biome in South America (Grooten & Almond 2018).

METHODS

In the Amazon Forest, water samples (500 ml each, in three replicates) were obtained from six sites within three main areas (A-C; Fig. 1, Appendices S1 and S2). In the Atlantic Forest, water and sediment samples (500 ml of water and 25 ml of sediment, in three replicates) were obtained from eight sites located in two valleys of the Caparaó National Park (D-E; Fig. 1, Appendices S1 and S2). Temperature and pH were recorded at each site in the Amazon. Mammal-specific universal primers targeting the mitochondrial 12S rRNA gene were used (Ushio et al. 2017). A total of 108 samples (including field, DNA extraction, and PCR blanks) were sequenced in two multiplexed runs on an Illumina MiSeq platform using the 2 x 150bp v2 chemistry. The workflow was conducted following the protocol described by Sales et al. (2019a; a more detailed description is included in Appendix S3).

Additional data regarding species' distributions in the Atlantic Forest were obtained through camera-trap surveys. Both valleys in the Caparaó National Park were surveyed with terrestrial and arboreal camera traps (Bushnell Trophy CamTM, USA; see Appendix S3).

RESULTS AND DISCUSSION

Approximately 1.3 million mammal reads were obtained after all the bioinformatic filtering (Amazon – 833623 reads; Caparaó National Park, Atlantic Forest – 109233 reads for water samples and 334593 for sediment samples). Only reads recovered for native mammals (919910 reads) were retained for downstream analyses.

Overall, we detected 28 molecular operational taxonomic units (MOTUs) from terrestrial and aquatic mammals, representing eight orders and 14 families (Appendix S4). Considering a threshold of >0.97 minimum identity, only 13 MOTUs could be assigned to the species level (Appendix S4). In the Amazon, six species were recovered, three of which are currently listed as threatened on the IUCN's Red List (2019) in different categories: the Endangered Amazon river dolphin *Inia geoffrensis*, the Vulnerable giant anteater *Myrmecophaga tridactyla,* and the Vulnerable lowland tapir *Tapirus terrestris*. Three least concern species were identified: *Thyroptera discifera* and *Rhynchonycteris naso* in the order Chiroptera, and the rodent *Toromys rhipidurus*. Detecting *Toromys* is significant as the genus is not known from the area. However, another congeneric species, *Toromys grandis*, is known from the Amazon River, not far from our study site (Abreu-Júnior et al. 2018). Only one MOTU was detected for each family (Fig. 1).

In Caparaó National Park, Atlantic Forest, nine families were detected using eDNA: five in the west side of the National Park (D) and nine in the east side (E; Fig. 1, Appendix S5). Of these, only seven could be assigned to species level (Appendix S4). Here, camera-trap surveys detected 17 species (and additional unidentified small mammal species), encompassing 12 families (Appendices S6 and S7). Combining the two non-invasive techniques, 15 families were detected overall (Table 1), six of them by both methods, three exclusively by eDNA metabarcoding, and six solely by the camera traps.

More MOTUs were retrieved for the families detected in the Atlantic Forest, suggesting the occurrence of several species of the same family in this area. For example, three MOTUs were recovered from the east side and two from the west side of the National Park for both Didelphidae and Cuniculidae. Camera trapping recorded three Didelphidae taxa (*Caluromys philander*, *Didelphis* sp.,

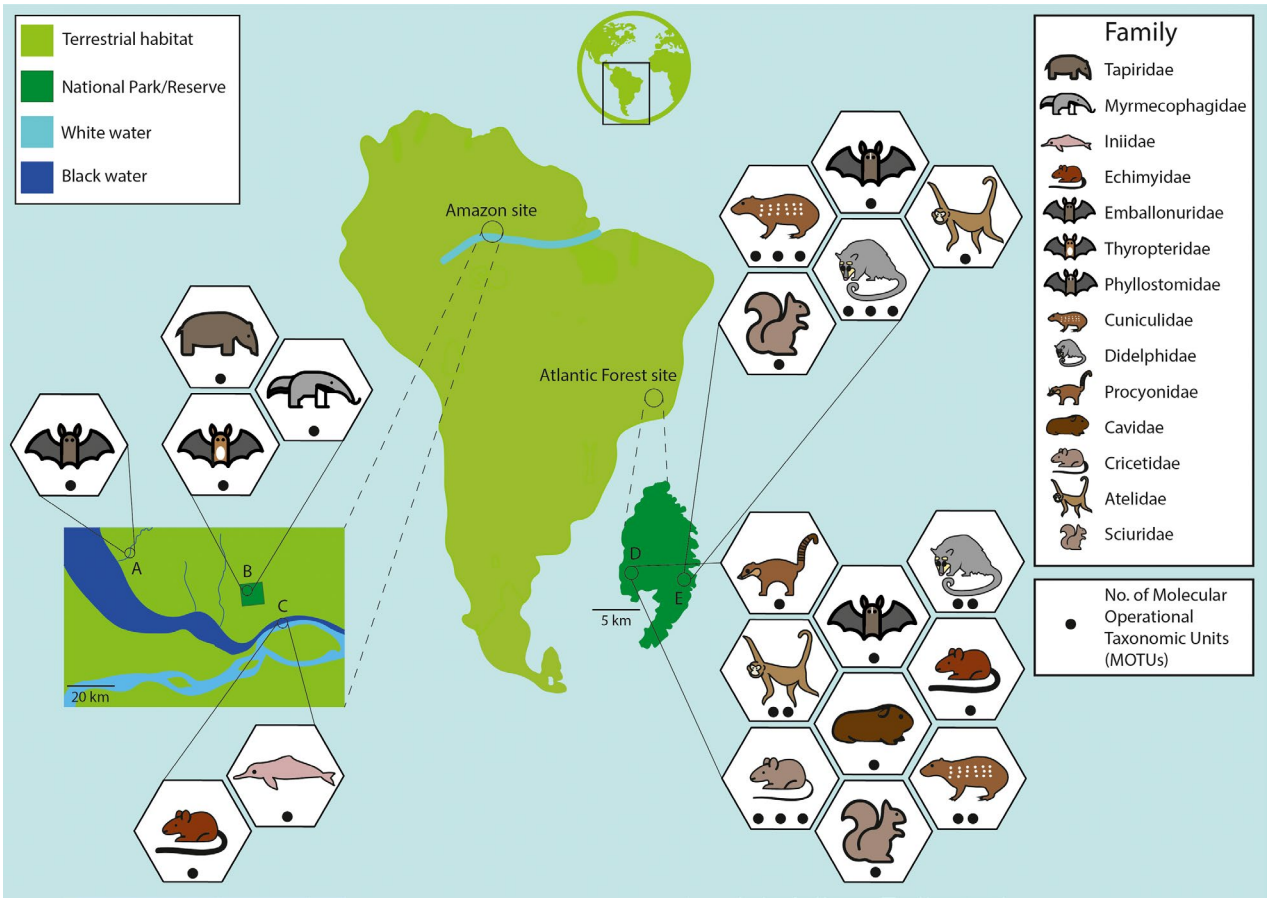

Fig. 1. Sampling areas for environmental DNA (eDNA) in the Amazon Forest (A-C) and Atlantic Forest (D-E) biomes in Brazil. The families recovered from eDNA metabarcoding in each area are represented by stylised drawings, and the number of molecular operational taxonomic units (MOTUs) recovered within each family is indicated by the number of dots

Table 1. Numbers (*n*) of species captured with camera traps and of Molecular Operational Taxonomic Units (MOTUs) captured with environmental DNA (eDNA) metabarcoding, for orders and families within Caparaó National Park, Atlantic Forest, Brazil. See Appendix S7 for a more extensive breakdown of camera-trap data

Order	Family	Camera (*n* species)	eDNA (*n* MOTUs)
Carnivora	Felidae	1	–
	Mustelidae	1	–
	Procyonidae	2	1
Chiroptera	Phyllostomidae	-	2
Didelphimorphia	Didelphidae	3	3
Pilosa	Myrmecophagidae	1	–
Primates	Atelidae	1	2
	Callitrichidae	1	–
	Cebidae	1	–
Rodentia	Caviidae	1	1
	Cricetidae	-	3
	Cuniculidae	1	3
	Echimyidae	2	1
	Erethizontidae	2	–
	Sciuridae	1	1

Philander frenatus), in accordance with the eDNA data. Only one species from the Cuniculidae (*Cuniculus paca*) recorded by camera traps is known to occur in the Caparaó National Park, Atlantic Forest, and the existence of three MOTUs for this family might be due to intraspecific genetic variability or cryptic species (within other groups also; Fig. 1). Cricetidae had three MOTUs in the west side of the National Park. Although this family was not identified by camera traps, several species are described for the Atlantic Forest, including endemic and recently described species (Gonçalves & Oliveira 2014). Furthermore, the Critically Endangered primate *Brachyteles hypoxanthus* was detected using eDNA, demonstrating the detection of arboreal mammals from water samples (e.g. Harper et al. 2019).

As a similar sampling effort was applied in both areas in this study, there is a need to consider what factors might explain the difference in the number of MOTUs recovered for each biome, particularly if we assume that mammalian alpha diversity should be at least as high in the Amazonian sampling sites as in the Caparaó National

Park, Atlantic Forest (see Costa et al. 2000). For example, all the families detected in the Atlantic Forest that were not detected in the Amazon Forest samples are known to occur in Area B of the Amazon Forest (Mendes Pontes et al. 2008). Degradation of DNA in water is one of the main factors reducing detectability over time and limiting temporal inferences. The sampled black waters in the Amazon have low pH (ranging from 3.85 to 4.27), whereas in the Caparaó National Park, Atlantic Forest, the reported pH values are above 6.5 (Rodrigues 2015). Acidic environments have higher decay and lower persistence rates of eDNA, due to the increased degradation of DNA via chemical hydrolysis (Seymour et al. 2018). Therefore, the eDNA recovered in the low pH waters of the Amazon might be derived from specimens that had recent contact with the water body. Mammal eDNA recovery depends not only on species presence but also on direct and indirect contact with the water system (Harper et al. 2019). The junction of the Negro and Solimões Rivers (Area C) has an enormous volume of water and possibly much time had elapsed since it flowed under the forest canopy; the other Amazonian streams (Area B; Fig. 1) are more similar in size to those in the Atlantic Forest. In the Amazon, all species and MOTUs were detected in a single replicate, except for the lowland tapir (detected in four replicates in three different streams). This species is known to defecate more frequently in water than on land (Tobler et al. 2010), so this may explain its higher rates of eDNA detection. In the Atlantic Forest, several MOTUs or species were recovered from multiple replicates and sites (Appendix S5), suggesting longer persistence of eDNA in this environment.

There is a clear limitation in terms of the available DNA sequences in public databases (e.g. GenBank) to match identified MOTUs to species. This issue has been highlighted in previous Neotropical eDNA studies for other taxonomic groups (Cilleros et al. 2019, Sales et al. 2019b). A 12S reference database exists for 164 Amazonian mammalian species in French Guiana (Kocher et al. 2017), and all Amazonian MOTUs were identified to species level here. However, this was not the case for the Atlantic Forest. This biome hosts more than 300 mammalian species (and more than 50% of medium and large species are considered at least Vulnerable; Souza et al. 2019). Therefore, for eDNA monitoring to be implemented in this biome, there is a clear need to generate reference DNA barcodes of a large proportion of the mammalian species present.

We demonstrated the potential of applying a cutting-edge non-invasive molecular approach to biodiversity assessments of Neotropical mammals (including highly threatened species). We recommend the use of eDNA metabarcoding alongside other non-invasive surveying methods in biodiverse regions (Harper et al. 2019, Sales et al. 2019a). However, significant challenges remain. To implement this method in the Neotropics, we need a better understanding of the ecology of eDNA within these variable environments, and more appropriate reference sequences for species identification in these biodiversity-rich and anthropogenically impacted biomes.

ACKNOWLEDGEMENTS

This project was partially funded by the University of Salford Internal Research Award awarded to CB, ADM, and IC. We are grateful to Vitor Borges for assistance in the field. The present study was carried out with all required permits (ICMBIO N. 54795-2, DEFRA 126191/385550/0).

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SUPPORTING INFORMATION AND DATA

Raw sequence data are available on figshare (https://doi.org/10.6084/m9.figshare.10045940 and https://doi.org/10.6084/m9.figshare.10045910).

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Appendix S1. Examples of four sampling areas for environmental DNA (eDNA): A = Santa Marta in the Atlantic Forest (Area E in Fig. 1); B and C = Acará (Area B in Fig. 1); and D = meeting of the waters, Amazon River (Area C in Fig. 1) in the Amazon.

Appendix S2. Co-ordinates and dates of eDNA sampling localities in the Atlantic Forest and Amazon. Information is provided on which samples were placed on each of two MiSeq sequencing runs.

Appendix S3. Detailed methods and references.

Appendix S4. Molecular operational taxonomic units (MOTUs) that were identified and their assignment to family, genus and species.

Appendix S5. Bubble graph representing presence–absence and categorical values of the number of reads retained (after bioinformatic filtering) for eDNA (water in blue and sediment in orange) from each family identified at each site (Areas D and E) in Caparaó National Park, Atlantic Forest.

Appendix S6. Collage of images representing examples of mammals captured from ground and canopy camera traps in Caparaó National Park, Atlantic Forest.

Appendix S7. Species captured by ground (G) and canopy (C) camera traps in Caparaó National Park, Atlantic Forest.