Final Evaluation Report

Your Details	
Full Name	Manuel Santiago Plata
Project Title	Assessing genetic diversity and gene flow for Neotropical otter (*Lontra longicaudis*) in the San Juan River Basin, Costa Rica
Application ID	33423-1
Date of this Report	07/31/2023

1. Indicate the level of achievement of the project's original objectives and include any relevant comments on factors affecting this.

Objective	Not achieved	Partially achieved	Fully achieved	Comments
1. Meetings and workshops (Government, NGO's, Universities)				As a result of COVID restrictions and the suspension of activities in Costa Rica, it was not possible to meet with professors and universities. However, we trained 22 people, including park rangers, locals, and volunteers, through five workshops distributed between Government Biological Stations and NGOs.
2. Fieldwork; first sampling period.				We utilised community-based monitoring to collect non-invasive genetic samples from faeces and anal jellies of otters and to adhere to the COVID regulations regarding sanitation in 2021. During the first sampling period, a group of 49 non-invasive genetic samples were collected in duplicate (DETs buffer, n=25; swabbing stored in ATL buffer, n=24). Additionally, the NGO named Caño Palma donated a set of 24 non-invasive genetic samples stored in silica.
3. Genetic analysis from the first and second sampling period				A single PCR multiplex of nine nuclear DNA microsatellite loci were amplified in duplicate for the first set of 74 non-invasive genetic samples collected during the first sampling period. As result of the genetic analyses for the first sampling period, a final PCR Neotropical otter multiplex of 10 nuclear DNA microsatellites loci was developed to conduct genetic analyses in all samples (N=174) collected after the first and second sampling period.
4. Data analysis from the first sampling period (population genetic parameters, and landscape genetic hypotheses)				During the first sampling period, nine nuclear DNA microsatellite loci were amplified. The amplification success rate for the first sampling period was 42%. The number of alleles per locus ranged from 1 to 7. After generating consensus genotypes and completing

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				matching, seven individuals were identified. In addition, a mean number of alleles per locus of 3.44, a mean observed heterozygosity (Ho) of 0.534 and an expected heterozygosity (He) of 0.552 were observed. Due to the small sample size, we were not able to test the landscape genetic hypotheses.
5. Dissemination of results from the first sampling period				We presented a poster during the 15th IUCN/SSC OSG International Otter Congress (September 19-23, 2022). We took part in an oral presentation webinar by the International Otter Survival Fund (IOSF) to commemorate World Otter Day on May 31, 2023. We plan to present our findings at the 30th TWS Annual Conference in Louisville, Kentucky from November 5-9, 2023, through an oral presentation.
6. Final data analyses and Landscape Genetic modeling				DNA amplification of a new multiplex of 10 loci yielded a successful amplification rate of 39%. Additionally, we can confirm that all loci were polymorphic and had a range of 3 to 10 alleles per locus. After consensus genotypes and completing matching, 24 individuals were identified, 11 were from Tortuguero National Park, and 13 were from the Sarapiquí river basin. We observed overall values of Ho=0.554 and He=0.640. The Tortuguero National Park exhibits slightly higher genetic diversity (Ho=0.583, He=0.658), as compared to the Sarapiqui river basin (Ho=0.526, He=0.621; see attachment, figure 1). Currently, additional sex identification analysis is ongoing. Unfortunately, we were unable to create a landscape genetic model due to the limited sample size. However, we are currently working on maximising PCR amplifications for partially amplified samples in order to increase

Objective	Not achieved	Partially achieved	Fully achieved	Comments
				the number of samples available for these analyses. We did evaluate genetic structure between the two watersheds and obtained a Fst value of 0.030 that shows moderate levels of gene flow.
7. Dissemination of the final results				We are currently focused on optimising PCR amplifications for partially amplified samples, as well as conducting further analysis for sex identification. Once all pending analyses are complete, we will share the final results at conferences, in my thesis and for publication.

2. Describe the three most important outcomes of your project.

a). We collected non-invasive genetic samples in duplicate using two methods (see attachment, figure 1): a) collection of ~500 µl of fecal material stored in dimethyl sulfoxide saline solution (DETs buffer), and b) external fecal swabbing stored in sodium dodecyl sulfate (ATL buffer). Through the utilisation of these sampling methods, we were able to demonstrate that fecal swabbing was the most successful field sampling and storage techniques for each sample type to enhance non-invasive genetic sampling for Neotropical otters (see attachment, figure 2). Additionally, we were able to compare the protocols used by different river otter species, specifically *Lontra longicaudis* and *Lontra canadensis* and demonstrate that fecal swabbing was the most successful method in multiple species and ecosystems (see attachment, figure 3)

b). Through our non-invasive genetic sampling methods for Neotropical otters using faecal DNA, we have identified polymorphic microsatellite loci for this region and gathered initial baseline data on the genetic diversity and genetic structure of Neotropical otters in Costa Rica. Additionally, we have created a genetic repository of the DNA samples we collected at the University of Idaho. We also created a map of the areas where we gathered the samples in the study area (see attachment, figure 4), and we developed a PCR multiplex that includes 10 nuclear DNA microsatellite loci for identifying Neotropical otters and assessing genetic parameters.

c). The project provided local volunteers and park rangers with training and workshops. The training was successful as it equipped them with new knowledge in non-invasive sampling methods, GPS unit management, identification of fresh otter feces, and understanding of the species' natural history and ecological function in rivers.

3. Explain any unforeseen difficulties that arose during the project and how these were tackled.

Throughout 2021, COVID restrictions and sanitary regulations in Costa Rica were substantial mainly because of the high prevalence of COVID positive cases throughout the country. We decided to reduce our study area and focus our sampling efforts on two regions associated with the San Juan River Basin (SJRB). Furthermore, considering Costa Rica's strict health regulations, we collaborated with park rangers and locals to carry out community-based monitoring.

The suspension of academic activities in the country changed all the plans to conduct workshops with students and researchers from the universities. We held meetings and workshops with park rangers, volunteers, and locals at field stations that were managed by the government and NGOs.

The high levels of precipitation was another challenge during the first sampling season. Although the zone is known for its high precipitation, that in 2021 was extraordinary compared to the 2022 rainy season. During May and June 2022, we carried out an intense sampling collection as the environmental conditions in the area were ideal during this period. Additionally, during our discussions with volunteers, locals, and park rangers, we agreed to only conduct non-invasive genetic sampling when environmental conditions were optimal (a minimum of 24 hours without any previous rain has passed).

4. Describe the involvement of local communities and how they have benefitted from the project.

We conducted training sessions and workshops for volunteers and park rangers in the local community who showed interest in our project. Due to COVID-19 cases and sanitary regulations in Costa Rica in 2021, community-based monitoring had limited success in terms of the number of samples collected. Nevertheless, we successfully imparted new knowledge to park rangers and locals through the sessions. They were trained in non-invasive sampling techniques, management of GPS units, identification of fresh otter faeces, and the natural history of the species and its ecological role in rivers.

As part of this project, we organised the first ever World Otter Day in Costa Rica and Central America. To make it happen, we conducted an Otter workshop and educational activities for 14 young students aged 7 to 12 from Tirimbina School. The workshop was done in partnership with the academic and research department of Tirimbina Biological Reserve. Tirimbina School is the only elementary school in the area and is crucial in educating local youth about the environment and raising awareness, as the students have frequent exposure to nature.

5. Are there any plans to continue this work?

Our intention is to continue working on this project. Currently, we are conducting analyses to optimise PCR amplifications for partially amplified samples. Our goal is to

include these samples in the final consensus genotypes for the second chapter of my thesis. In addition, as part of my thesis project, we are exploring a new research approach by incorporating DNA metabarcoding molecular diet analysis. We recently conducted a pilot analysis on six non-invasive otter samples (DETs buffer, n=3; swabbing stored in ATL buffer, n=3). We amplified a 75 bp fragment using a 12s rRNA primer and obtained some promising initial findings (see attachment, photo 5).

6. How do you plan to share the results of your work with others?

So far, the results from the first sampling season have been presented through a poster and an oral presentation at the 15th IUCN/SSC OSG International Otter Congress, as well as workshops organised by the International Otter Survival Fund (IOSF).

Additionally, I have been accepted to present an oral presentation at the 30th TWS Annual Conference in Louisville, Kentucky, from November 5-9, 2023. We look forward to sharing our results with the audience at the conference.

Our objective is to publish a minimum of two scientific articles using the funding provided by The Rufford Foundation: a) Assessing field collection and storage methods to improve fecal DNA genotyping and genetic data collection for Neotropical otters, and b) Evaluating demography, genetic diversity, and gene flow of Neotropical otters in Northern, Costa Rica.

7. Looking ahead, what do you feel are the important next steps?

As this project is a doctoral thesis, our next step will be to publish our findings in a manuscript that outlines our protocols for improving faecal DNA sampling techniques for Neotropical otters. The results of this chapter were made possible thanks to the Rufford Foundation's support for the first sampling period.

Additionally, we will finalise the PCR amplifications for partially amplified samples and complete the laboratory analyses for chapter two of my thesis, which is focused on genetic diversity, and gene flow of Neotropical otters in Northern, Costa Rica.

For my thesis project, we are exploring a new research direction that utilises a diet metabarcoding approach. Therefore, our next step would be to conduct molecular diet analysis on Neotropical otters and publish our findings in a third manuscript. To ensure we have enough resources, we are planning to request additional funding to cover the analysis of more samples and the inclusion of molecular diet analysis for Neotropical otters.

8. Did you use The Rufford Foundation logo in any materials produced in relation to this project? Did the Foundation receive any publicity during the course of your work?

We have attended congresses and workshops to share our results and have acknowledged The Rufford Foundation by displaying their logo. This will apply to any upcoming presentations in which we intend to share the results of the project.

Furthermore, any forthcoming article resulting from this project will acknowledge The Rufford Foundation.

9. Provide a full list of all the members of your team and their role in the project.

Team members
Lisette Waits: Dr. Waits was involved in the project as a molecular ecologist specialist, providing expertise in landscape genetics, conservation genetics, and population genetics. Additionally, Dr. Waits acted as a fieldwork assistant and served as the thesis advisor for the project leader.

David Roon: Dr. Roon is a faculty member at the University of Idaho who assisted with field work in the second sampling season.

Charlotte Foale & **Manuel Arias:** The Station Manager and Assistant Station Manager at the Caño Palma Biological Station provided us with all the necessary resources to carry out non-invasive genetic sampling.

Emily Khazan, Morgan Hughes & **Lianne Woudstra:** The Research Coordinators from The Caño Palma Biological Station assisted us at various times with training and coordinating volunteers for the sampling collection.

Charles Acuña: Administrator from La Selva Research Station. Charles provided us with all the necessary resources to carry out non-invasive genetic sampling within the research station and the Puerto Viejo River.

Danilo Brenes & **Greivin Salazar:** Laboratory Manager and fieldwork technician from La Selva Research Station. They conducted the non-invasive genetic sampling survey along the Puerto Viejo River for this project.

Mariela García & **Emmanuel Roja:** The Environmental Education Program leader and fieldwork technician at Tirimbina Biological Reserve were instrumental in providing us with the resources we needed for our project. They also carried out non-invasive genetic sampling at the Sarapiquí river and its tributaries.

Paul Foster: Director of Bijagual Ecological Reserve. Paul played an important role in the project by organizing the field technicians to collect non-invasive genetic samples from the Bijagual and Tirimbina rivers.

Wilmer Porras: Wilmer is a member of the community who has expertise in rafting and kayaking activities in the town of La Virgen-Sarapiquí. Wilmer's expertise in the river proved valuable as he worked as a fieldwork technician and logistics support to carry out non-invasive genetic otter sampling at Puerto Viejo and Sarapiquí rivers.

10. Any other comments?

At first, we planned to conduct a survey in all sub-basins that are part of the San Juan River Basin (SJRB). However, due to COVID regulations and restrictions in the country throughout 2021 forced us to modify our original sampling plan. As a result,

we decided to: a) train volunteers and park rangers in non-invasive genetic sampling to conduct community-based monitoring. After completing all meetings and training, we returned to the US for health security, and b) to reduce our study area and concentrate our sampling activities on two specific regions: the Sarapiqui river basin and the Tortuguero National Park. These regions are of great importance for conservation due to their abundant diversity of plant and animal life. However, the rise in economic and tourist activities in these areas may threaten the long-term survival of river otter populations.

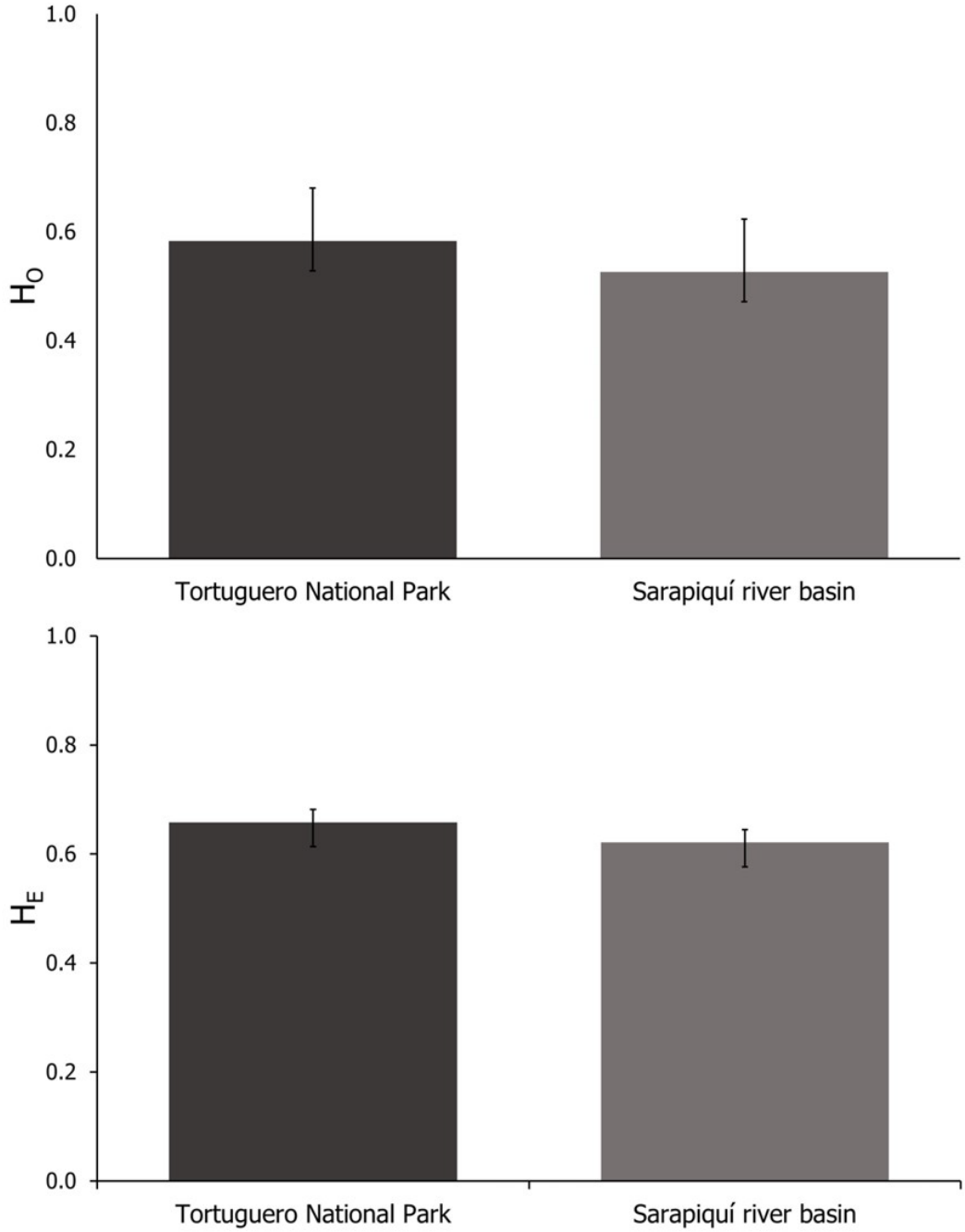

Figure 1. Genetic diversity of the Neotropical otter in Tortuguero National Park and the Sarapiqui river basin of Costa Rica. Observed heterozygosity (HO) and Expected heterozygosity (H_E).

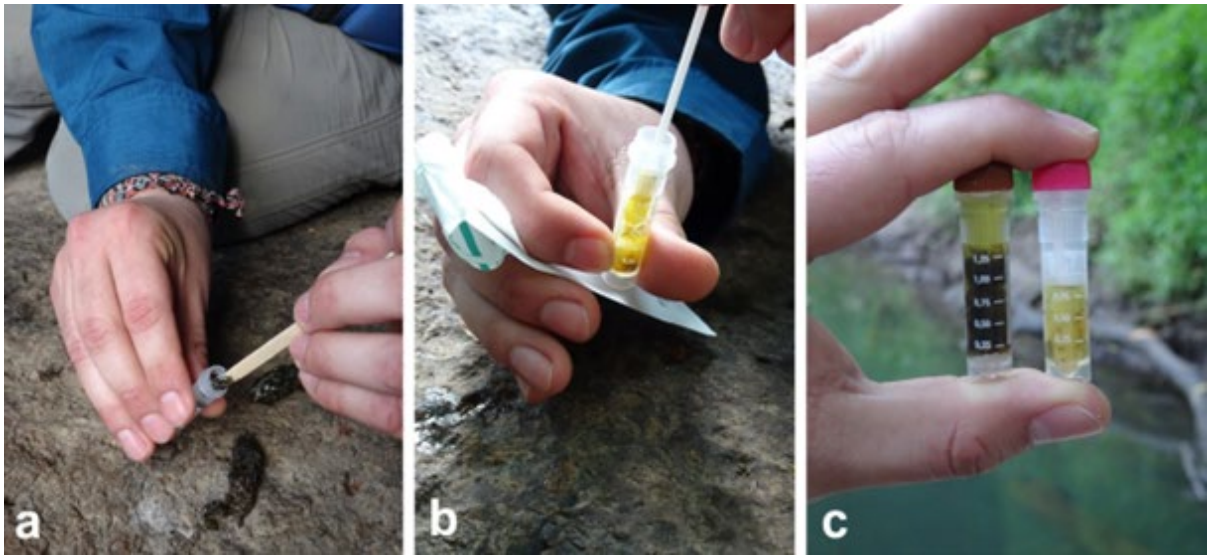

Figure 2. Field sampling and storage protocols; a) faecal material stored in DETs buffer, b) external faecal swabbing stored in ATL buffer, c) sample collected in duplicate.

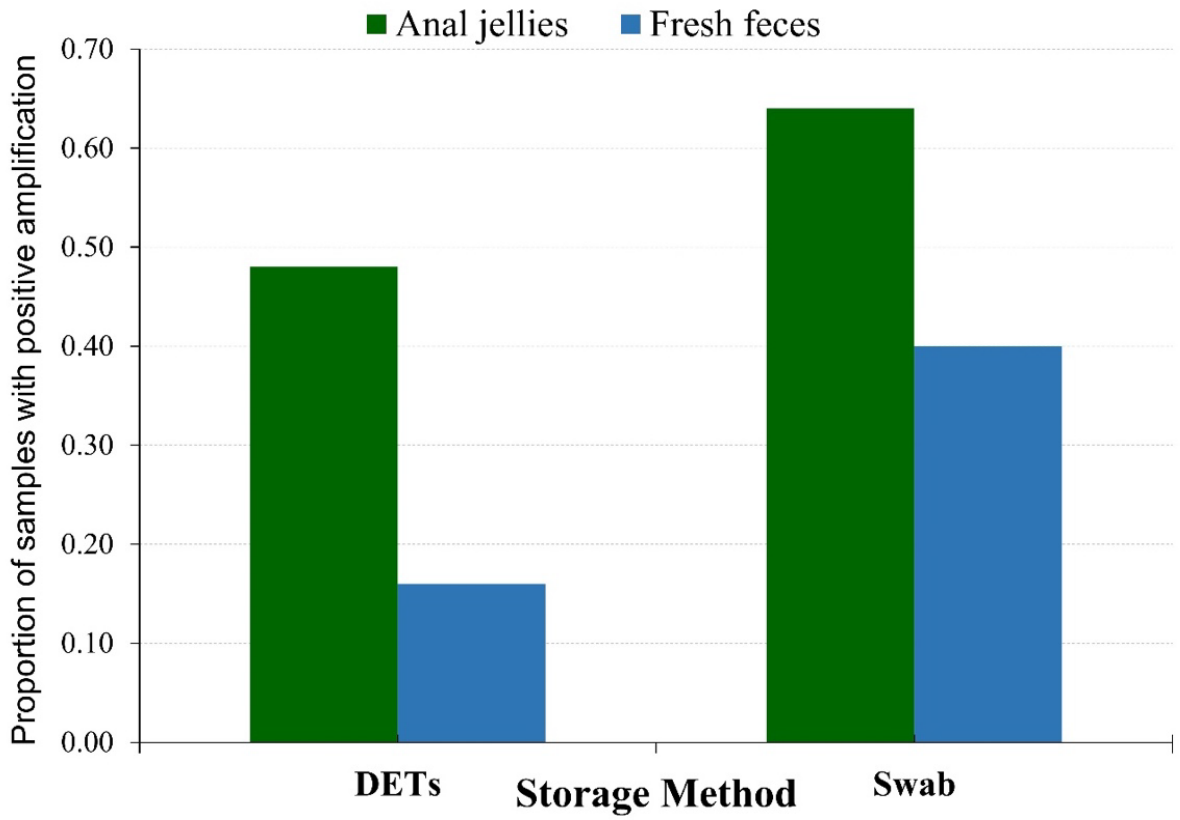

Figure 3. DNA amplification success rate by storage method and sample type for neotropical river otters in Tortuguero National Park, Costa Rica.

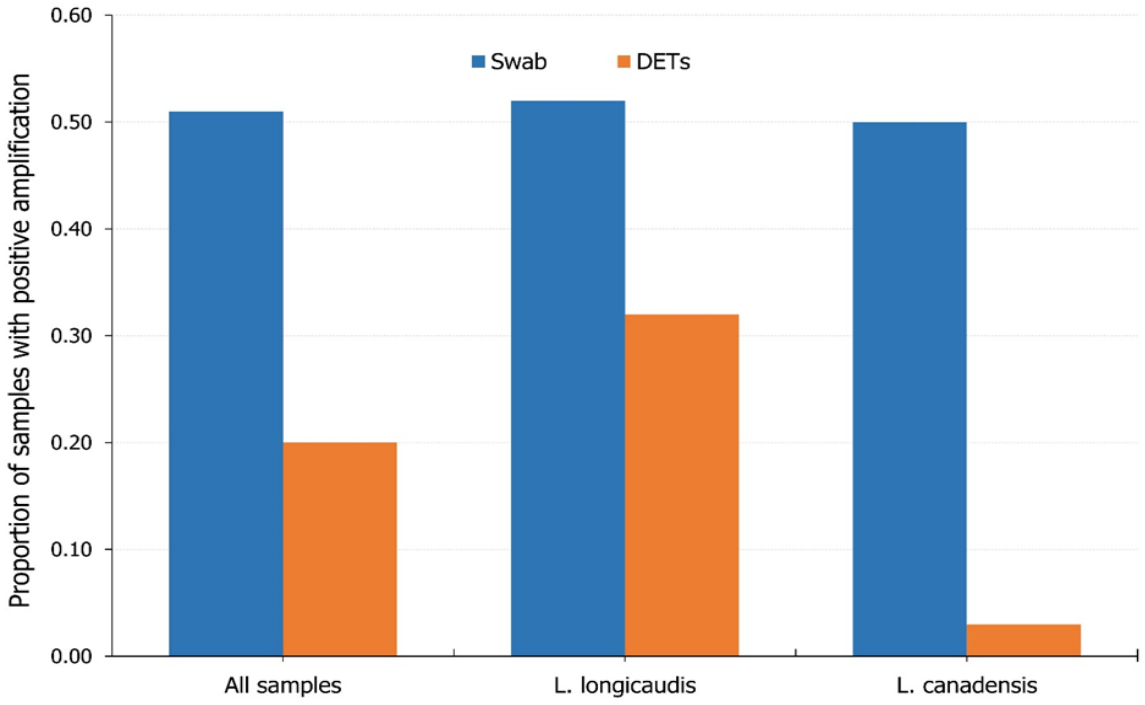

Figure 4. DNA amplification success rates for faecal and anal jelly samples collected and stored using two methods for Neotropical river otter (L. longicaudis) in Costa Rica and the North American river otter (L. canadensis) in South Dakota, USA.

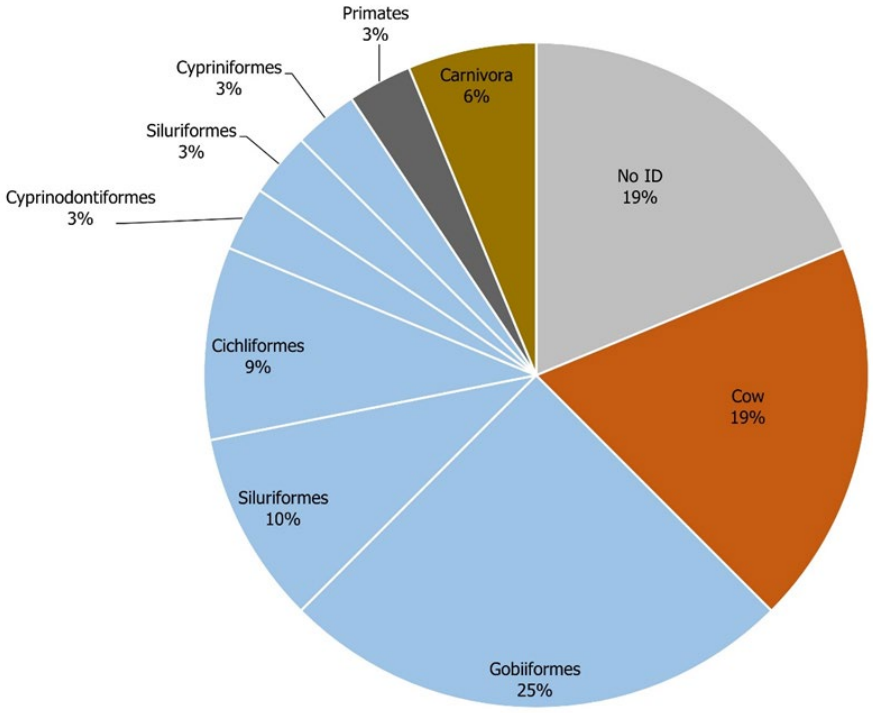

Figure 5. Preliminary results of diet metabarcoding of six non-invasive otter samples generated by amplifying a 75 bp fragment using a 12S rRNA primer and Illumina DNA sequencing.

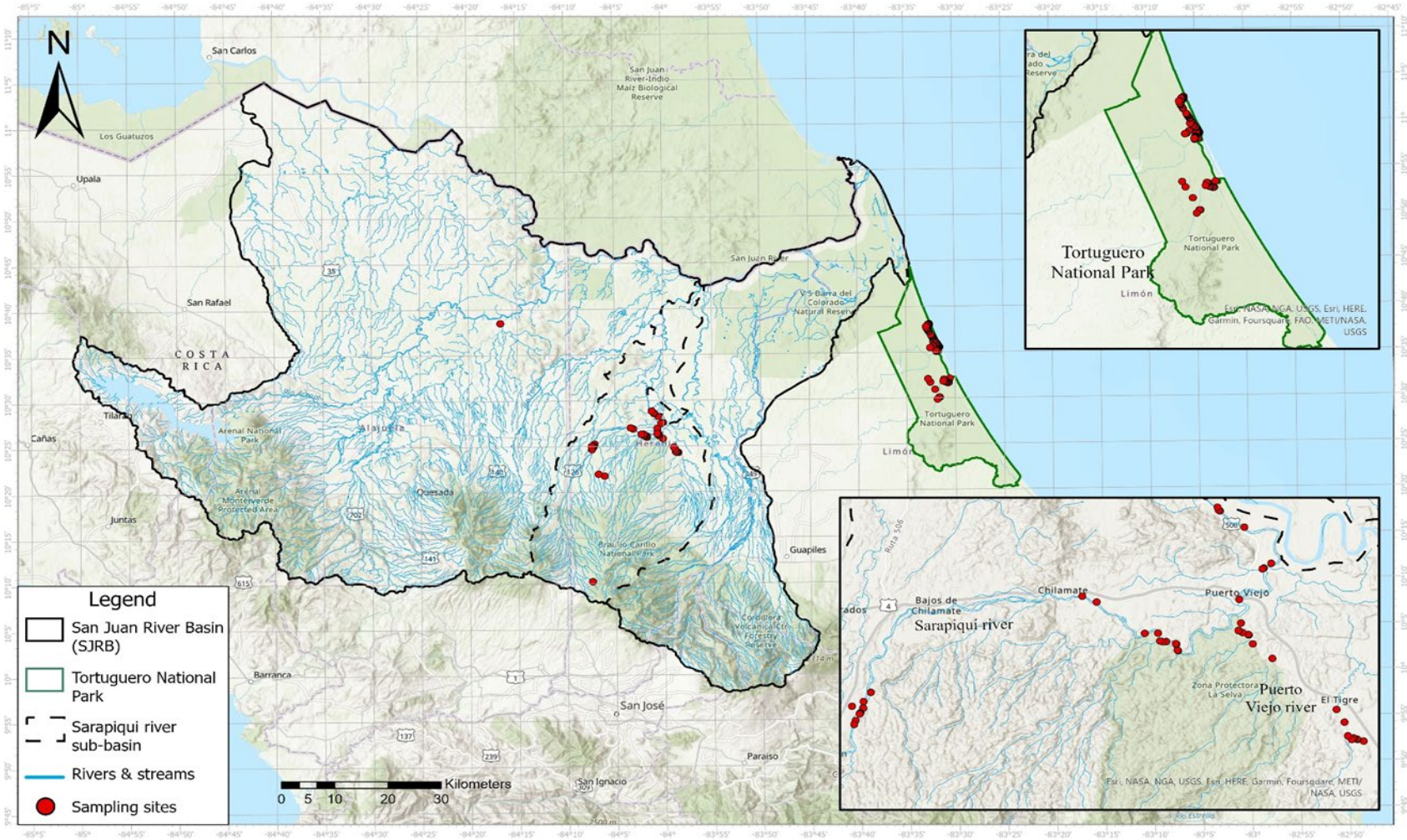

Figure 6. Location of the Sarapiqui River Basin and Tortuguero National Park in Costa Rica, along with the collection sites of non-invasive genetic samples from Neotropical otters.