Form 3-200-37

OMB Control No. 1018-0093
Expires 08/31/2020

RCVD OCT 02 2019

Department of Interior
U.S. Fish and Wildlife Service

Federal Fish and Wildlife Permit Application Form

U.S. Fish and Wildlife Service
Division of Management Authority
Branch of Permits, MS: IA
5275 Leesburg Pike
Falls Church, VA 22041-3803
1-800-358-2104 or 703-358-2104

Type of Activity

EXPORT/RE-EXPORT/IMPORT/INTERSTATE AND FOREIGN COMMERCE/TAKE OF ANIMALS (LIVE/ SAMPLES/PARTS/PRODUCTS) under the Convention on International Trade in Endangered Species (CITES) and/or the U.S. Endangered Species Act (ESA)

Complete Sections A or B, and C, D, and E of this application. U.S. address may be required in Section C, see instructions for details. **Instructions on how to make your application complete and help avoid unnecessary delays are attached.**

Section A: Complete if applying as an individual

1.a. Last Name		1.b. First Name	1.c. Middle Name/Initial	1.d. Suffix
2. Date of Birth (mm/dd/yyyy)	3. Telephone Number	3.a. Alternate Telephone Number	4. E-mail address	

Section B: Complete if applying on behalf of a business, corporation, public agency, Tribe, or institution

1.a. Name of business, agency, Tribe, or institution		1.b. Doing business as (DBA)	
Institute for the Conservation of Tropical Environments			
2. Tax identification no.		3. Description of business, agency, Tribe, or institution	
		Organization for conducting conservation research in Madag	
4.a. Principal officer Last name	4.b. Principal officer First Name	4.c. Principal officer Middle name/initial	4.d. Suffix
Wright	Patricia	C	
5. Principal officer title	6. Primary contact name		
Executive Director	M. Elise Lauterbur		
7.a. Business telephone number	7.b. Alternate telephone number	7.c. Business fax number	7.d. Business e-mail address
(217) 649-2566			lauterbur@gmail.com

Section C: All applicants complete address information

1.a. Physical address (Street address; Apartment #, Suite #, or Room #; no P.O. Boxes)				
N-203 Social and Behavioral Sciences Building, Stony Brook University				
1.b. City	1.c. State	1.d. Zip code/Postal code	1.e. County/Province	1.f. Country
Stony Brook	NY	11794-4310	Suffolk	USA
2.a. Mailing address (include if different than physical address; include name of contact person if applicable)				
2.b. City	2.c. State	2.d. Zip code/Postal code	2.e. County/Province	2.f.Country

Section D: All applicants MUST complete

1. Attach the **nonrefundable application processing fee** in the form of a check or money order payable to the U.S. FISH AND WILDLIFE SERVICE **in the amount of $100.** Federal, Tribal, State, and local government agencies, and those acting on behalf of such agencies, are exempt from the processing fee – *attach documentation of fee exempt status as outlined in instructions* [50 CFR 13.11(d)].

2. Certification: I hereby certify that I have read and am familiar with the regulations contained in ***Title 50 Part 13 of the Code of Federal Regulations*** and the other ***applicable parts in subchapter B of Chapter I of Title 50***, and I certify that the information submitted in this application for a permit is complete and accurate to the best of my knowledge and belief. I understand that any false statement herein may subject me to the criminal penalties of 18 U.S.C. 1001.

Patricia C. Wright 09/26/2019

Signature of applicant/Principal Officer for permit (No photocopied or stamped signatures) Date of signature (mm/dd/yyyy)

Please continue to next page

E. EXPORT/RE-EXPORT/IMPORT/INTERSTATE AND FOREIGN COMMERCE/TAKE OF ANIMALS (Live/samples/parts/products) (CITES and/or ESA)

Allow at least 90 days for the application to be processed. Applications for endangered species permits must be published in the Federal Register for a 30-day public comment period.

Complete all questions on the application. Mark questions that are not applicable with "N/A". If needed, use separate sheets of paper. On all attachments or separate sheets you submit, indicate the application question number you are addressing. If you are applying for multiple specimens, be sure to indicate which specimen you are addressing in each response.

NOTE: The import of live southern white rhinoceros from South Africa and Swaziland must meet specific CITES criteria for an import permit to be issued. If you are requesting authorization for the import of these species, please ensure that you respond to question 14 below.

Electronic submission of inventories, photographs, and receipts: Some applications contain extensive inventories and /or a large number of photographs or receipts. You may provide electronic versions of the documents. Such a submission will assist the processing of your application since it may reduce data entry by the U.S. Fish and Wildlife Service. If you wish to provide information electronically, once you have received an application number via the e-mailed acknowledgment letter, e-mail your information to Permits@fws.gov. Be sure to include the application number provided in the acknowledgment e-mail that will be sent to you when we receive your application.

☐ I will be submitting documents electronically.

1. Name and address where you wish the permit to be mailed, **if different from page 1**. If you would like expedited shipping, please enclose a self-addressed, pre-paid, computer-generated, courier service airway bill. If unspecified, all documents will be mailed via regular mail through the U.S. Postal Service.

2. Who should we contact if we have questions about the application (name, phone number, and e-mail)?

M. Elise Lauterbur, (217) 649-2566, lauterbur@gmail.com

3. Have you or any of the owners of the business (if applying as a business, corporation, or institution), been assessed a civil penalty or convicted of any criminal provision of any statute or regulation relating to the activity for which the application is filed; been convicted, or entered a plea of guilty or nolo contendere, for a felony violation of the Lacey Act, the Migratory Bird Treaty Act, or the Bald and Golden Eagle Protection Act; forfeited collateral; OR are currently under charges for any violation of the laws mentioned above?

☒ No ☐ Yes

If you answered "Yes" to Question 3, provide: a) the individual's name; b) date of charge; c) charge(s); d) location of incident; e) court, and f) action taken for each violation. Please be aware that a "Yes" response does not automatically disqualify you from getting a permit.

4. What activity are you requesting authorization to carry out (Indicate appropriate activities):

☐ EXPORT ☐ RE-EXPORT ☑ IMPORT ☐ TAKE (e.g., cull, lethal harvest)
☐ INTERSTATE COMMERCE ☐ FOREIGN COMMERCE

Note: Interstate Commerce permits authorize the sale of endangered and threatened species across State lines, but only for activities that will contribute to enhancing the propagation or survival of that species. Interstate commerce activities with wildlife require the buyer to obtain a permit prior to the sale or offer for sale.

5. For **EACH** animal/specimen involved in the proposed activity provide:

a. Scientific name (genus, species, and, if applicable, subspecies)	**b. Common name**	**c. Birth/ Hatch Date** *(mm/dd/yyyy)* (approximate of actual unknown)	**d. Wild or captive-born**	**e. Quantity**	**f. Gender** (male or female, if known), if	**g. Permanent markings**, if alive (e.g., tattoo, ID #, microchip #, scars)	**h. Type of sample or product** (e.g., blood, tissue, DNA)
EXAMPLE: *Pan troglodytes*	Chimpanzee						
See attached.							

6. The current location of the specimen(s) (address and country):

Name:

Address:

City:

State/Province:

County, Postal Code:

7. Recipient/Sender:

- If **export**, provide name and address of the recipient in the foreign country.
- If **re-export**, provide the name and address of the recipient in the foreign country.
- If **import**, provide name and address of the exporter in the foreign country.
- If **interstate or foreign commerce**, provide name and address of the proposed seller/supplier.

Name:

Address:

City:

State/Province:

County, Postal Code:

SOURCE OF SPECIMEN (answer question 8 or 9 for **EACH** animal/specimen involved, as appropriate).

8. For captive-bred animals or animal(s) from which the specimen(s) are/were obtained, provide a signed and dated statement from the breeder that includes the following:
   a. Scientific name (genus, species, and, if applicable, subspecies) and common name;
   b. Name and address of the facility where the animal was bred and born;
   c. Birth/hatch date (mm/dd/yyyy), and, if applicable, identification information;
   d. Location (name of facility, address, city, State, postal code) of parental stock;
   e. A statement that the animal was bred at the above facility;
   f. Documentation demonstrating the history of transactions (e.g., chain of custody or ownership of the animal).
9. For **EACH** animal/specimen **taken from the wild**, provide the following:
   a. Scientific name (genus, species, and, if applicable, subspecies) and common name;

See attached.

   b. Specific location of where, when, and by whom (name and address) the specimen was removed from the wild;

See attached.

   c. Purpose of removal and length or approximate length of time held in captivity. Discuss issues such as the method of collection, was the collection done as part of a larger study, were animals returned to the wild after sampling, and did any mortalities or injuries occur due to collection or holding;

See attached.

   d. If and how any remuneration, either financial or in-kind, was provided for taking or capturing animals or for the collection of samples.

See attached.

   e. Your efforts to use captive specimens (e.g., captive-born, captive-held), or parts thereof, in lieu of taking animals from the wild.

See attached.

   f. Copies of your foreign or domestic collecting permit, license, contract or agreement;
   g. Documentation showing that the specimen(s) was/were legally obtained by the applicant; and
   h. Copies of any applicable State, Tribal, Federal, or Foreign government permits or licenses that authorized the removal of this animal from the wild.

JUSTIFICATION FOR REQUESTED ACTIVITY.

10. Provide a detailed statement justifying the proposed activity, particularly the following:

    a. Describe the purpose of your proposed activity. For example, if the purpose is scientific research, attach a copy of your research proposal outlining the purpose, objectives, methods (e.g., specific information on survey/collection methods, sampling regime, equipment to be used), and whether similar work has already been done or is currently being done. If the purpose includes conservation education, provide copies of educational materials (e.g., handouts, text of signage or public presentations), and include the purpose and objectives of the proposed activity. If the purpose is for propagation for conservation purposes (including culling as part of herd management), provide a description of how the species will be propagated and the disposition of progeny, as well as long-term goals of the breeding program, how the breeding program is managed to maintain genetic vitality, and information on any cooperative breeding programs or agreements that are/will be established, including any future plans for re-introduction.

See attached.

    b. Description of the technical expertise of each person (please also include CV or resume), as it relates to the proposed activities. If the proposed activity involves live animals, include the experience of each animal caretaker working with the species.

See attached.

    c. Copies of contracts, agreements or other documents that identify persons involved and dates of activities for which authorization is being requested.

11. A statement on how the activities will **enhance or benefit the wild population** (e.g., in-situ and ex-situ projects).

See attached.

12. If live specimens are to be held in captivity as part of the proposed activity:

a. Provide a detailed description (e.g., size, construction materials, protection from the elements) and photographs or diagrams (no blueprints, please) clearly depicting the existing facilities **where the wildlife will be maintained**. If the specimens will be housed at multiple facilities, either immediately or within the next year, provide a full description of each facility. If you are unsure of which facilities may be receiving specimens (e.g., final decisions on placement have not been made), please indicate likely candidates and the mechanism that will be used to determine recipient facilities;

NA

b. A statement of the specific technical experience of CV or resume available to the recipient(s) for maintaining and propagating live specimens of the same or similar species;

NA

c. The number of years each species has been maintained at the facility;

NA

d. The number of births by year for each species for the last 5 years; and

NA

e. Mortalities at the facility with these or similar species in the last 5 years, causes of such mortalities, and steps taken to avoid or decrease such mortalities.

NA

IMPORTS, EXPORTS, OR RE-EXPORTS.

13. For shipment of LIVE specimens, the transport conditions for animals must comply with the CITES Guidelines for Transport of Live Animals or, in the case of air transport, with the International Air Transport Association (IATA) live animal regulations (contact airline for information). As such, describe:

a. The type, size, and construction of any shipping container; and

NA

b. The arrangements for watering or otherwise caring for the wildlife during transport.

NA

14. For import of live southern white rhinoceroses from South Africa and Swaziland, a determination that the importing facility meets the CITES "appropriate and acceptable destination" annotation must be made. Therefore, provide written documentation demonstrating that the proposed activity would promote *in situ* conservation of the species. **Note: *For any permit authorizing trade of live rhinoceroses under an "appropriate and acceptable destination" annotation, the rhinoceros horn from these animals may not enter commercial trade and the animal may not be sport hunted.***

15. **For import of LIVE CITES Appendix-I listed marine mammal species**, provide a copy of your FWS or NOAA Fisheries permit or authorization.

16. For import of CITES **Appendix-I listed species**, provide information to show the import is not for primarily commercial purposes as outlined in Resolution Conf. 5.10 .

17. For export of CITES **Appendix-I listed species**, provide a copy of the CITES import permit, or evidence one will be issued by the Management Authority of the country to which you plan to export the specimen(s). In accordance with Article III of the CITES treaty, it is required that import permits are issued before the corresponding export permit.

18. If the specimen is being **re-exported** (e.g., exporting a specimen that was previously imported into the United States), provide:

    a. A copy of the canceled CITES export or re-export document issued by the appropriate CITES office in the country from which the wildlife was imported (if applicable); and

    b. A cleared copy of Form 3-177, wildlife Declaration for Import (hard copy or electronic release); **OR**

    c. If you did not make the original import, provide a copy of the importer's documents outlined above and the invoice or other documentation that shows you acquired the wildlife from the original importer or history of transactions which demonstrate chain of ownership.

All international shipment(s) must be through a designated port. A list of designated ports (where an inspector is posted) is available. If you wish to use a port not listed, please contact the Office of Law Enforcement for a Designated Port Exemption Permit (form 3-200-2).

Directorate of the Management of Renewable Natural Resources and Ecosystems
No. 214-19/MEDD/SG/DGEF/DGRNE

RESEARCH AUTHORIZATION

Name: Patricia Wright

Capacity: Researcher

Accompanied by: Behak Weatherington, Ezzeldin Enan, Haja Rakotondrainibe, Jake Krauss, Amanda Du Bour, Ryan Rothman, Lianne Woudstra, Thomas Kelly, Tobias Gräble, Sina Feyer, Heninkaja Rasoaviarimanana, Pascal Rabeson, Velotsara Jean Baptiste, Dina Andrianoely, Georges Razafindrakoto, Mamitina Velonabison, Georges Rene Rakotonirina, Remi Rakotovao, Randrianasolo Laurent, Zakamanana Francois, Dominique Razafindraibe

Overseeing organization: Department of Biological Anthropology and Sustainable Development (MADD) BP906 Antananarivo (101)

Place: Near AP: Mahasoa, Mangarabaka, Ranomafana.

Duration: Six (06) months starting August 2019

Is authorized to perform research on:
"Proposal of a *Prolemur simus* translocation and reintroduction project to the forest of Talatakey of Ranomafana National Park"

Special mention of activities:
- Capture and release, placement of collars and following of collared *Prolemur simus* individuals, with choice of individuals to capture
- Evaluation of the health and measurement of captured animals
- Collection of fecal material and ectoparasites in Ivato forest
- Collection of biological samples from the captured animals (blood, fur, tissue, ectoparasites, fecal material) for subsequent analyses; behavioral observation in Ivato forest.

FORMAL INTRODUCTION OF PARK AND PROTECTED AREA ENTRY
NO PRODUCT DEVELOPMENT IS AUTHORIZED

Export: Three milliliters (3ml) per blood sample per individual and two (02) drops on filter paper per individual; three (03) samples of tufts of fur per individual; three (03) tissue samples per individual; ten (10) samples of dead ectoparasites specimens and on hundred (100) samples of fecal material.

Obligations of permit holder:
- Negotiate for site or forest access with the managers and/or management committee, where applicable
- Report sampling activities to the regional representative of DREDD, or his representative
- Present project aims to the Regional Director of the Environment and Sustainable Development Office, and/or DREDD, before any research is conducted in the research locality, pursuant to note **n. 394-10/MEF/SG/DGF/DVRN/SGFF of May 18 2010**
- Provide to the Department for Management of Renewable Natural Resources and Ecosystems four (04) copies IN FRENCH of the preliminary research report at the end of the expedition and the final

report with research results no later than one year after the expedition, in hard copy and electronic versions
- Respect all forest regulations, any irregularity from this Research Authorization is considered to be a forest crime
- For all transport of collected products (animal and plant), have a Report of Observation of collected samples by the CEDD concerned, and transport authorization issued by DREDD if the transport is out of the region, and submit a copy to DGRNE
- For all exports: submit one copy of the transport authorization to DGRNE and one other to the export file

Additions:
DREDD – Atsimo Atsinanana. Vatovavy fitovinany (Malagasy, refers to the location)
CEDD: concerning
Concerned municipalities
For control and monitoring
DGEF
For control and monitoring
MADD
For the report

Antananarivo, Aug. 6 2019
Director of Management of Renewable Natural Resources and Ecosystems
(Signed and sealed by RAKOTOARIDERA Rantonirina)

DIRECTION DE LA GESTION DES RESSOURCES NATURELLES RENOUVELABLES ET DES ECOSYSTEMES

N° 214/MEDD/SG/DGEF/DGRNE

AUTORISATION DE RECHERCHE

NOM ET PRENOM : Patricia Wright

FONCTION : Chercheur

ACCOMPAGNE DE : BekahWeatherington, EzzeldinEnan, HajaRakotondrainibe, Jake Krauss, Amanda Du Bour, Ryan Rothman, Lianne Woudstra, Thomas Kelly, Tobias Gräble, Sina Feyer, Heninkaja Rasoaviarimanana, Pascal Rabeson, Velotsara Jean Baptiste, Dina Andrianoely, Georges Razafindrakoto, Mamitina Velonabison, Gerges Rene Rakotonirina, Remi Rakotovao, Randrianasolo Laurent, ZakamananaFrancois, Dominique Razafindraibe.

ORGANISME TUTEL : Mention Anthropobiologie et Developpement Durable (MADD) BP 906 Antananarivo (101)

LIEU : Hors AP : Mahasoa, Mangarabaka, Ranomafana.

DUREE : Six (06) mois à partir d'Août 2019.

EST AUTORISE (E) A FAIRE DES RECHERCHES SUR :

« Proposition de projet de reintroduction et translocation de *Prolemur simus* dans la forêt de Talatakely du Parc National de Ranomafana. »

MENTION SPECIALE D'ACTIVITES

-Capture aver relâche, pose de collier et suivi des individus marqués de *Prolemur simus*, choix des individus à capturer

-Evaluation de la santé et mensuration des animaux capturés.

-Collecte de matières fécales et ectoparasites dans la forêt d'Ivato

-Collecte d'échantillons biologiques sur les animaux capturés (sang, poils, tissus, ectoparasites, matières fécales.) pour des analyses ultérieures ; observation de comportements dans la forêt d'Ivato.

INTRODUCTION FORMELLE DE S'INTRODUIRE DANS LES PARC NATIONAUX ET LES AIRES PROTEGEES

AUCUN DEVELOPPEMENT DE PRODUITS N'EST AUTORISE

EXPORTATION : Trois millilitres (3ml) d'échantillons de sang par individus et deux (02) gouttes sur papier filtre par individus ; trois (03) échantillons de touffes des poils par individus ; trois (03) échantillons de tissus par individus ; dix (10) échantillons des specimens mort d'ectoparasites et cent (100) échantillons de matières fécales.

OBLIGATIONS DU TITULAIRE :

- Négocier avec les gestionnaires et/ou comité de gestion des sites ou forêts transférées pour y accéder, le cas échéant
- **Effectuer une restitution au niveau Régional avec le procès verbal y afferent signé par le DREDD ou son répresentant**
- Faire viser la présente par la Direction Régionale de l'Environnement et du Développement Durable et/ou DREDD concernées avant toute descente sur terrain conformément à la note **n° 394-10/MEF/SG/DGF/DVRN/SGFF du 18 Mai 2010** de la localité de recherche
- Remettre à la Direction de la Gestion des Ressources Naturelles renouvelables et des Ecosystèmes, en quatre (04) exemplaires EN FRANÇAIS, le rapport préliminaire à la fin de sa mission et le rapport final avec les résultats des recherches au plus tard UN an après la mission, en versions papier et électronique.
- **Respecter la réglementation en matière forestière et toute irregularité aux mentions de l'Autorisation de Recherche est considéré comme un délit forestier**
- Pour tout transport de produits de collecte (faune et flore), avoir un procès-verbal de constatation des collectes effectuées par le CEDD concerné et autorisation de transport délivré par DREDD si le déplacement se fait en dehors de la région et remettre une copie au DGRNE
- Pour toute exportation : remettre une copie du dépôt au DGRNE et une autre au dossier d'exportation

AMPLIATIONS :
DREDD Atsimo Atsinanana, Vatovavy Fitovinany
CEDD concernees
Communes concernees
« Pour contrôle et suivi »
DGEF
« Pour contrôle et suivi »
MADD
« Pour le rapport »

Antananarivo, le 06 AOUT 2019

LE DIRECTEUR DE LA GESTION DES RESSOURCES NATURELLES RENOUVELABLES ET DES ECOSYSTEMES

RAKOTOARIDERA Rantonirina

Le Chef Cantonnement de l'Environnement de l'Ecologie et des Forêts

Vu au passage à Ifanadiana

RANDRIANDRAKOTO [illegible]

Vu au passage à Fort-Carnot
LE SECRETAIRE
ARRIVEE
du 13 Août 2019
CIREDD
RAJANOMEHA Soso Charle


SAMILLE

REQUEST TO USE ANIMALS – GENERAL INSTRUCTIONS

University of Akron
Kent State University
Northeast Ohio Medical University
Summa Health System
Youngstown State University

PROTOCOL COMPLETION

Each of the animal care programs at the institutions listed above uses the following "Request to Use Animals" (protocol) and the related "Annual Review" and "Modification" forms for all animal work involving live animals. Consult your Institutional Animal Care and Use Committee (IACUC) Coordinator to access the forms at your institution. Other forms may be required by each institution.

You must complete a new protocol form for each submission. Answer all questions that apply in a manner comprehensible to the layperson and define discipline specific terminology and abbreviations the first time they are used. Enter all responses in the answer boxes provided. For Yes/No questions and those that are not applicable (N/A), check the box or insert an "X" to the right of the appropriate response. Guidance in responding to the questions is provided by resting the cursor over the highlighted word in each section or by reviewing the "Comment" box in the margin of each page (Word version).

PROTOCOL SUBMISSION

Submit the completed documents electronically as an email attachment along with other required forms (e.g., hazardous substances), to the IACUC Coordinator at each institution at which any animal work will occur. Consult the Institutional Animal Care and Use Committee (IACUC) Coordinator at your institution to determine institution specific submission requirements and processing procedures. Only word processed (minimum font size of 11 point) submissions will be accepted. The "Investigator Assurance" and "Participant Qualifications" pages must be included with the submission. Final approval cannot be granted until all signed signature pages are received.

ANNUAL REVIEWS

Animal use protocols must be renewed annually using the "Annual Review – Request to Use Animals" form. Protocols continuing longer than three years must be resubmitted in their entirety using the complete "Request to Use Animals" form prior to the three year anniversary of the original protocol. Although submission timelines may vary by institution, continuing protocols must be submitted at a time sufficiently prior to the expiration date to allow adequate time for IACUC review. Animal work covered by protocols that are not approved by the IACUC prior to their expiration date will be suspended until a new protocol is approved.

MODIFICATIONS

Any proposed changes to the animal work described in an approved protocol must be reviewed and approved by the IACUC before they are initiated. Submit any proposed changes to the IACUC on the "Modification – Request to Use Animals" form.

GENERAL

The information requested on the "Request to Use Animals" and related documents is needed to enable the IACUC to fulfill its regulatory requirement to review all research, teaching, and testing activities involving live vertebrate animals. Although the information provided will be treated confidentially by each of the IACUC's to the extent permitted by law, this document may be made available to the general public in response to Ohio Open Records Act requests filed with public institutions. Responses that are both professional and comprehensible to the layperson are encouraged. Feel free to contact the IACUC Coordinator or other designated IACUC spokesperson at your institution for advice in completing the form.

For IACUC Use Only
Category: C Special Considerations: J Protocol #: 482 AT 19-06
rev 1 Sub 8 July 2019

REQUEST TO USE ANIMALS

1. PROTOCOL SUMMARY

1. A. Protocol Title:

1. B. Principal Investigator's Institution:

Kent State University

1. C. Facility(ies) where animals will be housed: *If animals will be housed in a facility not listed below, please identify the location under "OTHER LOCATION".*

- UNIVERSITY OF AKRON ☐
- KENT STATE UNIVERSITY
  - Cunningham ☐
  - Kent ☐
  - Tuscawaras ☐
- NEOMED ☐
- SUMMA HEALTH SYSTEM ☐
- YOUNGSTOWN STATE UNIVERSITY
  - Cushwa ☐
  - DeBartolo ☐
  - Ward Beecher ☐
- OTHER ☒

OTHER LOCATION (Institution, building & room OR geographic location for field studies):

Ranomafana National Park, Madagascar

1. D. Source of funding for the project:

INTERNAL ☐
EXTERNAL ☒

For external awards, identify the agency(ies) and award number(s).

National Science Foundation, Biological Anthropology Program
Recommended for funding

1. E. Anticipated start date:

January, 2020

1. F. Expected animal use over the three year approval period: *Summarize all animal use by species.*

Species	Number	Source
Hapalemur aureus	10-15	Ranomafana National Park

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

1. G. If this protocol is a continuation of a previously approved protocol, indicate the protocol number and provide a brief summary of the progress made to date. *Your response is limited to the space provided.* **N/A:**

Previous protocol number:

Brief summary of progress/results:

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

1. H. Project overview. *The response MUST be in lay terminology and understandable to a person with no scientific background.*

(1) Describe the medical condition, scientific question, or teaching value that is being addressed and its importance.

Cyanide is a poison known for its ubiquity among plants and low threshold for toxicity. Nonetheless, three species of bamboo lemur in and around Ranomafana National Park, Madagascar, focus most of their feeding time on various parts of Malagasy giant bamboo, which exposes the golden bamboo lemur (*Hapalemur aureus*) to 12-50 times their estimated lethal dose of cyanide on a daily basis. In addition to addressing the decades-old puzzle of how these lemurs are able to tolerate high level of this poison, this study stands to provide unique insights into the toxicology of cyanide, which has a history of application by militaries, terrorist organizations, and other malevolent entities. By elucidating how a primate such as *H. aureus* can avoid cyanide's effects, this study may inform the development of therapies, antidotes, or prophylactics to cyanide exposure in humans.

(2) List the goals of the project.

1. Create a reference-quality whole-genome assembly for the critically endangered golden bamboo lemur, *Hapalemur aureus*. This entails sequencing most of one specimen's genome to an averaging sequencing depth of 50-60 reads per site and piecing these reads together into long stretches, or scaffolds, in order to reconstruct entire chromosomes or otherwise long sections of the animal's genome.
2. Compare the gene expression profile of whole blood for *H. aureus* to that of *Lemur catta*, the closest relative that does not consume such a toxic diet
3. Analyze the levels of cyanide, thiocyanate, and other small molecules in the blood serum of several *H. aureus*

(3) Provide a chronological <u>summary</u> of the animal use from the beginning of the project through its end. *A lay description of the experimental design can be used as the response IF it addresses the intent of the question. <u>Do not</u> provide detailed descriptions of the procedures here.*

Co-PI Morgan Chaney will travel to Ranomafana National Park in Madagascar. There, he and a team of trained or licensed professionals will tranquilize a number of free-ranging *Hapalemur aureus* and draw approximately 6 mL of blood per animal. The animals will not be removed from the exact site where they are anesthetized and will be released back into that specific microhabitat upon regaining consciousness. The blood samples will be processed immediately after harvesting and placed in frozen storage until eventual analysis back in the United States. These samples will be used to complete Goals 2 and 3.

Also while at Ranomafana, co-PI Chaney will take postmortem tissue from a previously deceased *H. aureus* that is currently in ultracold storage there. This will be used to complete Goal 1.

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

2. DESCRIPTION OF PROCEDURES INVOLVING LIVE ANIMALS

Please review all parts of this section before answering because there are separate parts for specific types of animal use. Each part will expand to accommodate the response. Mark N/A for sections that do not apply.

2. A. Animal Identification:

Indicate how animals will be identified. Multiple methods may be selected.

CAGE CARD	☐	COLLAR/TAG	☐
EAR PUNCH/NOTCH	☐	EAR TAG	☐
INDELIBLE MARKER	☐	MICROCHIP	☐
TATTOO	☐	OTHER (describe below)	☐

Describe the identification procedure if it involves penetration of the skin. Toe clipping is discouraged and, if it is used, a justification must be provided.

In order to avoid resampling individuals, a semi-permanent and non-toxic marker (e.g., dye, paint) will be applied to each animal's fur when they are sedated.

2. B. Breeding: N/A: X

Describe the breeding scheme that will be used. Indicate weaning age of offspring.

2. C. *Genotyping:* N/A:

Describe the method used to genotype the animals. Include the amount of tissue taken, age of animals, method of analgesia, and method of instrument sterilization.

To accomplish our first objective in Section 1.H.2, we will use tissue (likely spleen or liver) from a deceased infant *H. aureus* that is currently in -80°C storage at Centre ValBio. This animal died of natural causes during a previous field season. We will extract genomic DNA from this tissue, isolate high molecular weight DNA from these extracts, and submit these processed samples to an external facility for high-throughout (or "next generation") sequencing on an Illumina platform with upstream microfluidic partitioning using the 10X Chromium sequencing platform.

Our second objective in Section 1.H.2 will be accomplished by extracting total RNA from the blood samples drawn from live, free-ranging, adult *H. aureus*. This total RNA will be shipped on dry ice to an external facility, where it will be further processed and sequenced on an Illumina platform.

2. D. Experimental manipulations: N/A:

List and describe in detail all nonsurgical experimental manipulations carried out on live animals. Euthanasia is to be described in 2.F. The response must include a statement of the known or expected impact of each procedure on animal well-being.

Groups of *H. aureus* will be located in Ranomafana National Park using the aid of staff workers from Madagascar National Parks. Telazol (5-6 mg/kg body weight) will be injected by intramuscular injection. This will be delivered by a pneumatically fired dart fired by an experienced marksman, who will be aiming for the anterior/lateral thigh in order to target the quadriceps femoris muscle. Darted animals will be caught in stretchers after the Telazol takes effect.

While sedated, two blood samples of ≥3 mL each will be drawn from each animal into vacuum tubes by the co-PI, an experienced veterinarian, and his/her assistant. Opportunistic measurements will also be taken of the animal's weight, as well as other non-invasive metrics (e.g., anatomical length measurements). The animal will be treated with an antiparasitic medicine. As mentioned herein, the animals' vital sign will be monitored continually and they will be marked by a semi-permanent marker in order to prevent resampling from the same animal at a later time.

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

2. E Surgical manipulations. N/A: X

2.E.(1) Description of surgical procedures:

Describe each surgical procedure under a separate heading. Procedures that are performed on the same animal at the same time may be described as one procedure. IF more than four different surgeries are planned, then similar ones may be combined into a single response.

Surgical procedure #1:

Is the surgical procedure a survival procedure? **Yes:** **No:**

Describe the procedure in detail. Include the pre-operative preparation of the animal, a description of the aseptic technique and how instruments and implantable devices are sterilized. The response must include a statement of the known or expected impact of the procedure on animal well-being.

Surgical procedure #2:

Is the surgical procedure a survival procedure? **Yes:** **No:**

Description of procedure (instructions as above):

Surgical procedure #3:

Is the surgical procedure a survival procedure? **Yes:** **No:**

Description of procedure (instructions as above):

Surgical procedure #4:

Is the surgical procedure a survival procedure? **Yes:** **No:**

Description of procedure (instructions as above):

2.E.(2) Multiple major survival surgery:

Does this project involve multiple major survival surgeries in the same animal? **YES:** **NO:**

If so, provide a justification.

2. F. Anesthesia/Sedation. N/A:

List the procedures that require anesthesia or sedation individually below and describe the anesthetic regimen used for each. If multiple procedures use the same anesthetic regimen, then they can be combined into one response.

Anesthesia/sedation procedure #1:

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

Identify the procedure requiring anesthesia or sedation. List all drugs (including neuromuscular blocking agents) used as part of the anesthetic/sedative regimen; include the dose, route of administration and indicate the frequency of repeat dosing. If animals will be anesthetized with inhalants, indicate the percentage of anesthetic gas, any auxiliary gases used, oxygen flow rate and ventilatory parameters (for mechanically ventilated animals).

Telazol (5-6 mg/kg body weight) will be injected by intramuscular injection. This will be delivered by a pneumatically fired dart.

Describe the procedures and equipment used to monitor the depth of anesthesia and animal well-being. If neuromuscular blocking agents are used, include techniques that are reliable in paralyzed animals.

During the procedure, the individuals will be monitored once every five minutes. A veterinarian will be present to monitor the following clinical parameters: heart rate, appearance, respiratory rate, and body temperature.

Describe the supportive measures to assure animal well-being while under anesthesia.

The potential adverse effects of administering Telazol are excessive salivation and drying of the eyes. In order to counter the excessive salivation, atropine (.54 mg/kg body weight) can be administered, and to counter any desiccation of the eyes an ophthalmic ointment will be applied. While under anesthesia, it is always a possibility that larger complications can occur such as depressed breathing or depressed heart rate, but these can be countered by monitoring vital rates (see above). All procedures will be conducted with a veterinarian present.

The potential adverse effect of drawing blood is that there is the potential for infection at the site of venipuncture. A topical antibiotic solution will be applied to the site of the blood removal to prevent possible infections.

The only other major risk posed by all other procedures is stress due to handling, which will be minimized by gentle handling, the wearing of PPE, close monitoring of each individual, and the ability to inject additional Telazol if an animal shows signs of waking up. The animal will be expected to wake up from the Telazol after 30-45 minutes of sedation.

Anesthesia/sedation procedure #2:

Identify the procedure requiring anesthesia/sedation and describe the anesthetic regimen as indicated above.

Procedures and equipment used to monitor the depth of anesthesia and animal well-being:

Supportive measures:

Anesthesia/sedation procedure #3:

Identify the procedure requiring anesthesia/sedation and describe the anesthetic regimen as indicated above.

Procedures and equipment used to monitor the depth of anesthesia and animal well-being:

Supportive measures:

Anesthesia/sedation procedure #4:

Identify the procedure requiring anesthesia/sedation and describe the anesthetic regimen as indicated above.

Procedures and equipment used to monitor the depth of anesthesia and animal well-being:

Supportive measures:

2. G. Building(s) and room number(s) where the procedures will take place:

Nonsurgical Procedures:

Surgical Procedures:

2. H. Postprocedural care and monitoring:

1) Describe the post-procedural care and monitoring for both surgical (after recovery from anesthesia) and nonsurgical procedures. Identify the parameters being monitored and the frequency and duration of monitoring for each study related procedure. Include how records of the care will be maintained and their location.

The animals will be monitored during recovery from the Telazol at the location where they were sedated until they awake. During the transitional period between sedation and full consciousness, the lemurs will be gently handled to reduce stress and given water to prevent dehydration. As mentioned in Section 2F, the animal may be given small doses of atropine and ophthalmic ointment to counteract possible negative side effects of the Telazol. Everything will be done in the presence of a veterinarian.

2) Identify by title who will conduct the care and monitoring.

KSU graduate student
Executive Director of Centre ValBio (30 years' experience in similar methods of sedation)
Malagasy veterinarian and his/her assistant

*3) List any analgesics or other medically related pharmaceutical agents that animals may receive. Include **a)** dose, **b)** route of administration **c)** frequency of administration, and **d)** duration of therapy.*

Ivermectin (dose of 0.2 mg/kg BW), delivered once by subcutaneous injection

4) List the criteria that will be used to determine that relief from pain or distress is needed and how the adequacy of that relief will be assessed.

Because the lemurs will experience momentary and minimal pain during this procedure, analgesia is not necessary.

5) List the humane endpoints that will be used to euthanize an animal or otherwise remove an animal from a study.

N/A

2. I. Disposition of animals:

Describe the method of euthanasia including the name, dose, and route of administration of any pharmaceutical agents used. Describe the method(s) that will be used to confirm death. Animals euthanized by an overdose of carbon dioxide must undergo a secondary method of euthanasia to confirm death. If animals will not be euthanized, describe their disposition.

N/A

2.J. Chemical/compound administration to live animals

Are all of the chemicals (e.g., test compounds, receptor agonists/antagonists, labeling compounds, anesthetics, analgesics, euthanasia agents, etc.) administered to live animals commercially available pharmaceutical preparations intended for animal or human use?

Yes: X **No:**

If not, then complete the following for each product.
Identify the chemical/compound and describe how it is prepared and stored to assure appropriate purity, sterility and suitability for administration to animals. Indicate the shelf life of the prepared product.

Are all of the chemicals/compounds listed above pharmaceutical grade? **Yes: X** **No:**
If not, then list them and provide a justification for not using a pharmaceutical grade preparation.

For IACUC Use Only Category:	Special Considerations:	Protocol #:

3. SPECIAL CONSIDERATIONS

Mark N/A for sections that do not apply.

3.A. Food/ fluid restriction: N/A: X

*If the study involves scheduling access to food or fluid OR restricting food or fluid intake beyond that associated with a routine overnight pre-procedural fast or weight control, then describe **a)** the amount and time of the restriction, **b)** expected impact on animal well-being, and **c)** criteria for removal of the restriction.*

Describe the record-keeping associated with ongoing restrictions. Indicate where the records will be maintained. At a minimum animal weights must be documented once weekly and food/water consumption noted daily.

3. B. Prolonged restraint: N/A: X

*If the project involves more than routine restraint of conscious animals for brief periods, then describe: **a)** the restraint, **b)** its duration and frequency, **c)** how animals will be conditioned to it, and **d)** how frequently animals will be observed while restrained.*

Provide a justification for the restraint.

3. C. Immunologic adjuvants: N/A: X

If the project involves the use of immunologic adjuvants (e.g., Freund's adjuvant, RIBI adjuvant) complete the following.

	First Injection	*Second Injection*	*Subsequent Injections*
Adjuvant			
Anatomic site of injection & route			
Number of sites			
Volume per site			
Time interval between injections			

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

3. D. Dog exercise: **N/A: X**

If the project involves the use of dogs, indicate if any animals will be exempted from the dog exercise program and include the duration of the exemption and a justification for it. If there are no exemptions, enter "no exemptions".

3. E. Environmental enrichment for primates: **N/A: X**

If the project involves the use of nonhuman primates, indicate if any animals will be exempted from the environmental enrichment program for primates and include the duration of the exemption and a justification for it. If there are no exemptions, enter "no exemptions".

3. F. Housing or enrichment restrictions: **N/A: X**

If the project involves the single housing of animals of a social species OR exemption from normal environmental enrichment, then describe and provide a justification for the restriction.

3. G. Hazardous material use: **N/A: X**

If the project involves the administration of any potentially hazardous materials to live animals, complete the following for each material and attach the appropriate hazardous material form(s) required by the institution at which the work will take place.

Name of hazardous agent(s):

Select the appropriate classification of hazard(s)

CARCINOGEN	☐	INFECTIOUS AGENT	☐
RADIOACTIVE ISOTOPE	☐	RECOMBINANT NUCLEIC ACID	☐
TOXIN	☐	HUMAN TISSUE/CELLS	☐
OTHER	☐		

Describe the potential health effects of the hazard and list the possible routes of exposure hazard:

Number of animals receiving material:

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

3. H. Genetically modified animals: N/A: X

If the project involves the use, breeding, or creation of genetically modified animals, complete the following for each genotype.

List the animals by genotype and describe the known or expected impact of the associated phenotype on animal well-being:

Describe the measures to relieve or manage pain or distress related to each phenotype that is associated with an adverse impact on animal well-being:

Will any new genetically modified animals be created in the project? **Yes:** **No:**

If so, describe the monitoring associated with the new line to assure adequate provision of humane animal care. Previously undescribed phenotypic conditions that negatively impact animal well-being must be reported to the IACUC:

3.I. Animal housing outside of main animal facility: N/A: X

If animals will be maintained outside of the main animal facility longer than 12 hours for USDA covered species or longer than 24 hours for all others, then complete the following.

Identify the building, room number, species, and number of animals to be housed. Indicate the duration of housing.

Provide a justification for the extramural housing.

Has the IACUC previously approved the location? **YES:** **NO:**

3.J. Field studies: N/A:

If the project involves the use of animals in a field setting, complete the following.

Identify the occupational health and safety issues associated with studying the species in the wild.

Lemurs are primates, and as such there is some potential for zoonotic disease. This will be lessened through the use of protective equipment such as nitrile gloves and surgical masks. In addition to routine immunizations, the KSU graduate student performing the fieldwork will be additionally immunized against hepatitis A, hepatitis B, typhoid, yellow fever, rabies, and polio; this variety of immunizations is aligned with CDC recommendations for Americans traveling to Madagascar.

Describe the potential impact of the study on native populations of the species being studied and others that may be affected by the study.

There will be a minimal or negligible impact on native populations because the anesthetized animals will regain consciousness and be released in the same habitat where they were initially sedated.

List and attach the permits and other necessary permission documents that are needed to carry out the study.

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

Attached is a letter of permission and endorsement from the Executive Director of Centre ValBio, the field station in Ranomafana where this study will be conducted.

3.K. Procedures performed at a supplier location: N/A: X

If animals will undergo experimental or surgical procedures at a supplier's location, complete the following and attach a statement from the supplier confirming IACUC approval of the procedure. Identify the procedure and supplier's Public Health Service Animal Welfare Assurance number and USDA registration number (as applicable).

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

4. CLASSIFICATION OF PROCEDURES ACCORDING TO LEVEL OF PAIN AND/OR DISTRESS

Mark the appropriate category for each animal procedure and identify the procedure(s) in the spaces provided. List the number of animals in each pain category in the box provided. If individual animals will undergo procedures in multiple pain categories, then include them in the tabulation for the highest pain category.

☒ - **Category C** - Procedures that involve no more than momentary or slight pain or distress.

List procedures:

Animals will be located in the forest with the help of a trained guide from Madagascar National Parks, and a stretcher will be suspended directly below the animal's position in the forest canopy. The animal will be darted with a sedative (Telazol; see below) and caught in the stretcher. At this point, blood samples and measurements will be taken while the animal is unconscious. The animals will not be moved from the site where they are darted, and they will be released back into this habitat.

Number of animals in category C:

10-15 Hapalemur aureus

☐ - **Category D** - Procedures that may cause more than momentary or slight pain or distress for which appropriate analgesia, anesthesia or tranquilization is provided.

List procedures:

Number of animals in category D:

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

☐ - **Category E** - Procedures that may cause pain or distress which are not relieved by analgesia, anesthesia, or tranquilization.
List procedures:

Number of animals in category E:

For Category E procedures: Provide a detailed scientific justification for withholding analgesia, anesthesia, and tranquilization.

5. ALTERNATIVES TO THE USE OF ANIMALS AND PAIN OR DISTRESS PRODUCING PROCEDURES

Provide a written narrative description of the methods and sources that were used to determine that suitable alternatives to the use of animals and to the pain or distress producing procedures described in the protocol are not available. Provide an explanation for alternatives that were identified but deemed unsuitable. Literature searches must include a) databases searched, b) the date of the search, c) the years covered by the search (minimum 10 years), and d) the search strategy including keywords used. At least two acceptable information sources must be used. The response must address the three R's: Replacement models, Refinements in technique, and Reduction in animal numbers. Information sources that are commonly used include http://www.pubmed.gov, http://agricola.nal.usda.gov, http://www.nal.usda.gov/awic, and specifically for teaching activities, http://oslovet.veths.no.

We queried NCBI's PubMed database (on June 22, 2019) and Clarivate Analytics's Web of Science database (on July 3, 2019) using the following key terms: cyanide intoxication, animal model, cyanide detection, non-invasive, and RNA sequencing sample size

A recent replacement animal model is a line of mice bred by Sabourin et al. (2016: *Int J Toxicol* 35*(5)*). The acute oral lethal dose (as LD_{50}) in this line of rodent was between 9.9 and 11.8 mg of potassium cyanide per kg body weight. Such doses are very minimal in comparison with the 1850 µmol of HCN consumed by *H. aureus* (Ballhorn et al., 2009: *Am J Primatol* 71), which is equivalent to about 190 mg/kg in this species. Thus, no replacement animal model would be suitable for this study.

Most non-invasive methods for detecting cyanide exposure involve assaying the urine (e.g., Vaz et al., 2012: *PLoS One 7(4)*), but such studies have already been conducted on this population by others (Yamashita et al., 2010: *Am J Primatol* 72; M.E. Lauterbur, unpublished). The previous detection of such high levels of cyanide and thiocyanate in these lemurs' urine justifies follow-up work testing their blood for these compounds (as well as novel gene-expression profiles) because of the intensity of the levels documented by previous authors.

There is currently no biological consensus on how many individual samples would be sufficient to control for false discovery in RNA-sequencing experiments, but it is uncontroversial that the adding of more individuals (or biological replicates) will improve statistical power (Liu et al., 2014: *Bioinformatics* 30*(3)*).

Although some comparative RNA-sequencing work has used as few as four individuals per species (Perry et al., 2012: *Genome Res* 22*(4)*), we aim to maximize statistical power in our analysis. Furthermore, because it is likely that multiple *H. aureus* populations within Ranomafana National Park will be sampled as part of this protocol, we see the potential for a second use of these samples in documenting the population genetics of this critically endangered species as part of a future study.

6. JUSTIFICATION FOR THE USE OF ANIMALS

6. A. Provide a rationale for involving animals.

The bloodstream of these animals was identified as a tissue of special interest for this study because it plays an important role in the toxicodynamics of cyanide poisoning. In this case, the animal is known to consume cyanogenic foods nearly exclusively for a large part of the year and its behavioral response shows no adverse effects. Thus, it is warranted to directly measure levels of cyanide, its byproducts, and detoxification substrates in the blood.

6. B. What is the basis for selecting the species that you have chosen?

No other species is known to be able to tolerate such high doses of cyanide, with the possible exception of the closely related greater bamboo lemur (*Prolemur simus*).

6. C. Number of animals requested:

Provide a justification for the number of animals requested. Identify the species, genotypes, strains, and/or stocks of animals. Include other descriptors as relevant (e.g., age or weight, gender, timed pregnant). For research protocols, list the experimental and control groups and indicate the number of animals in each. Include the statistical justification, or other basis, for selecting the number requested. If a research protocol includes the use of animals solely for training (i.e., the training does not occur as part of the experimental use of animals), then include the expected number of animals to be used for training. Animals used for training can be justified by documenting the expected number of persons to be trained and the number that can be trained per animal.

We hope to sample at least two *H. aureus* populations within Ranomafana National Park, and part of this study includes RNA sequencing experiments to detect differentially expressed (DE) genes. Previous work (Williams et al., 2014, *Curr Protocols Hum Genet* 38*(1)*) recommends sequencing at least three biological replicates per population, assuming 30 million reads per sample, in order to detect DE genes between populations. Other work (Liu et al., 2014: *Bioinformatics* 30*(3)*) rounds out this recommendation by showing that the number of DE genes increases with the addition of up to four more replications (i.e., seven in the study by Liu et al., 2014). The statistical power, with an FDR of 0.05 and 30 million reads per sample, roughly converges in experiments with more than 5 replicates (Liu et al., 2014). Thus, we have decided 10-15 animals between two populations would be sufficient for our purposes.

6. D. Provide written assurance that the use of animals described in this protocol does not unnecessarily duplicate previous experiments.

A previously published study measured cyanide or its metabolite thiocyanate in the urine (Yamashita et al., 2010, *Am. J. Primatol.* 72); and more recent, but unpublished work by a collaborator (M. Elise Lauterbur, Stony Brook U.) has measured the precise levels of these compounds in the urine using mass-spectrometric methods. No work up to this point, however, has attempted to measure these compounds in the bloodstream of these animals.

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

7. HOUSING AND HUSBANDRY

7. A. Indicate the approximate number of animals to be housed at one time and approximate duration of housing.

None. No animals will be housed because they will be only briefly sedated before being released back into their original habitats.

7. B. If rodents are to be housed, is there a preference as to the type of caging (i.e., plastic, wire-bottom, microisolator or other) OR the number of animals per cage? **YES: NO:**

If yes, please specify. Note that the use of wire-bottom cages or single housing of animals requires a justification.

N/A

7. C. Will a light cycle other than the standard 12 hours light/12 hours dark be necessary for any of the animals on this protocol? **YES: NO: X**

If yes, please specify the light cycle(s) and indicate the group(s) of animals that will require it.

7. D. Will the animals on this protocol have any special temperature or humidity requirements? **YES: NO: X**

If yes, please describe.

7. E. Will the animals on this protocol require a special diet or special water? **YES: NO: X**

If yes, please identify the product, the number of animals receiving it, and who will prepare and administer it.

7. F. Will the animals on this protocol require any other special housing, care, environmental conditions, or other considerations? **YES: NO: X**

If yes, please describe.

7. G. Will it be necessary to house animals after they have received any hazardous materials (refer to Part 3.G.)? **YES: NO: X**

If yes, please identify the material, the number of animals, and the duration of housing. Describe how the housing cages and room will be identified to alert personnel that a hazard is present.

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

8. PROTOCOL APPROVAL

Click "Choose Institution" to select the institution to which the protocol will be submitted.

Protocol approval is indicated by the signatures of the institution-specific individuals identified below. The individuals signing confirm that they have reviewed the protocol and find it to be in compliance with applicable animal care and use regulations and institutional policies.

Kent State University

Approval Signatures:

_____ Date _____
Facility Director

_____ Date _____
Department Chair/Research Director

_____ Date _____
IACUC Member

_____ Date _____
Attending Veterinarian

_____ Date _____
IACUC Chairperson

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

INVESTIGATOR ASSURANCE

By signing below I/we agree to:

A. Employ procedures that will avoid or minimize discomfort, distress, and pain to animals, consistent with sound research design.

B. Comply with the protocol as approved by the Institutional Animal Care and Use Committee (IACUC) and to obtain the consent of the IACUC before implementing any changes to the protocol.

C. Comply with the policies of the IACUC of the institution at which this work is conducted, the National Research Council Guide for the Care and Use of Laboratory Animals, the Public Health Service Policy on Humane Care and Use of Laboratory Animals, the regulations of the Animal Welfare Act and other applicable federal, state and local regulations governing the use of animals in research, teaching, and testing.

D. Maintain adequate records of all animal experimentation procedures.

E. The provision of emergency veterinary care including euthanasia by the attending veterinarian or his/her designee for animals showing evidence of unbearable pain, distress, or illness with the understanding that an effort will be made to contact me or my designee prior to the initiation of any treatment.

Principal Investigator:

Name: Anthony Tosi

Department: Anthropology

Email address: atosi@kent.edu

Telephone number: (330)672-5121

Signature Anthony L Tosi

Date: July 3, 2019

Co-Investigator:

Name: Morgan Chaney

Department: Anthropology

Email address: mchaney1@kent.ed

Telephone number: (330)672-4028

Signature Morgan Chaney

Date: 7/3/2019

Co-Investigator:

Name:

Department:

Email address:

Telephone number:

Signature _____

Date: _____

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

PARTICIPANT QUALIFICATIONS

Complete this form for the principal investigator, each co-investigator, and each of the individuals who may participate in the animal work described in the protocol. By signing below the participant acknowledges that he/she has read the protocol and agrees to comply with it.

NAME: Dr. Anthony J. Tosi, Ph.D.

TITLE: Associate Professor

EMAIL: atosi@kent.edu

List the participant's responsibilities on the protocol.

PI Tosi will not participate in the field component of this protocol. He will provide oversight and guidance during the phase of the research following co-PI Morgan Chaney's return from the field.

Describe the participant's experience and/or qualifications relevant to the responsibilities on the protocol. If the participant has no relevant experience then check here ☒ and identify below who will be responsible for training.

EXPERIENCE/QUALIFICATIONS:

Dr. Patricia C. Wright will provide training for co-PI Chaney.

DESCRIPTION OF FORMAL ANIMAL CARE AND USE TRAINING:

TITLE OR DESCRIPTION OF TRAINING	LOCATION	DATE OF TRAINING
CITI Basic Biosafety Training	http://www.citiprogram.org/	21-Feb-2019
CITI Animal Biosafety	http://www.citiprogram.org/	28-June-2019

Anthony J Tosi

PARTICIPANT SIGNATURE

July 3, 2019

DATE

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

PARTICIPANT QUALIFICATIONS

Complete this form for the principal investigator, each co-investigator, and each of the individuals who may participate in the animal work described in the protocol. By signing below the participant acknowledges that he/she has read the protocol and agrees to comply with it.

NAME: Morgan E. Chaney

TITLE: Ph.D. Candidate

EMAIL: mchaney1@kent.edu

List the participant's responsibilities on the protocol.

Co-PI Chaney will assist the veterinarian and Madagascar National Parks staff in the sedation of the lemurs and the collection of blood samples.

Describe the participant's experience and/or qualifications relevant to the responsibilities on the protocol. If the participant has no relevant experience then check here ☒ and identify below who will be responsible for training.

EXPERIENCE/QUALIFICATIONS:

Dr. Patricia C. Wright will provide training for co-PI Chaney.

DESCRIPTION OF FORMAL ANIMAL CARE AND USE TRAINING:

TITLE OR DESCRIPTION OF TRAINING	LOCATION	DATE OF TRAINING
CITI Basic Biosafety Training	http://www.citiprogram.org/	14-Feb-2019
CITI Animal Biosafety	http://www.citiprogram.org/	21-June-2019
Responsible Conduct of Research (BMS 61000)	Northeast Ohio Medical University	Fall, 2015

PARTICIPANT SIGNATURE

7/3/19
DATE

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

PARTICIPANT QUALIFICATIONS

Complete this form for the principal investigator, each co-investigator, and each of the individuals who may participate in the animal work described in the protocol. By signing below the participant acknowledges that he/she has read the protocol and agrees to comply with it.

NAME: Dr. Patricia C. Wright, Ph.D.

TITLE: Executive Director and Founder of Centre ValBio

EMAIL: patchapplewright@gmail.com

List the participant's responsibilities on the protocol.

Supervision of darting and sample collection during experimental procedures. Dr. Wright will also train co-PI Chaney while he is visiting Centre ValBio.

Describe the participant's experience and/or qualifications relevant to the responsibilities on the protocol. If the participant has no relevant experience then check here ☐ and identify below who will be responsible for training.

EXPERIENCE/QUALIFICATIONS:

Dr. Wright has 30 years of experience in collecting measurements from lemurs in Ranomafana National Park following protocols using sedative darts and similar methods outlined here.

DESCRIPTION OF FORMAL ANIMAL CARE AND USE TRAINING:

TITLE OR DESCRIPTION OF TRAINING	LOCATION	DATE OF TRAINING
Workshops on the sedation and capture of lemurs, trained by Dr. Kenneth Glander, Ph.D. and several DVMs	Duke University	1987-1990
Hands-on training in the sedation and capture of lemurs, trained by Dr. Kenneth Glander, Ph.D. and several DVMs	Ranomafana National Park, Madagascar	1987-1990

[See attached letter of support]

PARTICIPANT SIGNATURE

DATE

26. Sep. 2019

CITES import permit application
Institute for the Conservation of Tropical Environments

5. For **EACH** animal/specimen involved in the proposed activity provide:

a. Scientific name	**b. Common name**	**c. Birth/Hatch Date**	**d. Wild or captive-born**	**e. Quantity**	**f. Gender**	**g. Permanent markings**	**h. Type of sample or product**	**9.b. Date sampled**
Prolemur simus	Greater Bamboo Lemur	Estimated 2018	Wild	4	Male	Microchip #0A01750051	Blood (2mL tubes)	Aug. 1 2019
Prolemur simus	Greater Bamboo Lemur	Estimated 2018	Wild	1	Male	Microchip #0A01750051	Buffy Coat (2mL tube)	Aug. 1 2019
Prolemur simus	Greater Bamboo Lemur	Estimated 2018	Wild	1	Male	Microchip #0A01750051	Hair (plucked, stored in 2mL tube)	Aug. 1 2019
Prolemur simus	Greater Bamboo Lemur	Estimated 2018	Wild	1	Male	Microchip #0A01750051	Plasma (2mL tube)	Aug. 1 2019
Prolemur simus	Greater Bamboo Lemur	Estimated 2018	Wild	2	Male	Microchip #0A01750051	Protein Saver Card	Aug. 1 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	4	Female	Microchip #0A02011231	Blood (2mL tubes)	Aug. 1 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	Microchip #0A02011231	Buffy Coat (2mL tube)	Aug. 1 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	Microchip #0A02011231	Hair (plucked, stored in 2mL tube)	Aug. 1 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	Microchip #0A02011231	Plasma (2mL tube)	Aug. 1 2019
Prolemur	Greater	Unknown	Wild	3	Female	Microchip	Protein	Aug. 1

simus	Bamboo Lemur	(adult)				#0A02011231	Saver Card	2019
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	3	Male	Microchip #0A01750201	Blood (2mL tubes)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	1	Male	Microchip #0A01750201	Buffy Coat (2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	1	Male	Microchip #0A01750201	Hair (plucked, stored in 2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	1	Male	Microchip #0A01750201	Plasma (2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	2	Male	Microchip #0A01750201	Protein Saver Card	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	3	Female	Microchip #0A01752151	Blood (2mL tubes)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	Microchip #0A01752151	Buffy Coat (2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	Microchip #0A01752151	Hair (plucked, stored in 2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	Microchip #0A01752151	Plasma (2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	2	Female	Microchip #0A01752151	Protein Saver Card	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	3	Female	Microchip #0A01750829	Blood (2mL tubes)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	1	Female	Microchip #0A01750829	Buffy Coat (2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	1	Female	Microchip #0A01750829	Hair (plucked, stored in	Aug. 2 2019

							2mL tube)	
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	1	Female	Microchip #0A01750829	Plasma (2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	2	Female	Microchip #0A01750829	Protein Saver Card	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	3	Female	Microchip #0A01752351	Blood (2mL tubes)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	*Prolemur simus*	Buffy Coat (2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	*Prolemur simus*	Hair (plucked, stored in 2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	*Prolemur simus*	Plasma (2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	*Prolemur simus*	Protein Saver Card	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	2	Female	*Prolemur simus*	Blood (2mL tubes)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	3	Male	Microchip #0A01751807	Blood (2mL tubes)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	1	Male	Microchip #0A01751807	Buffy Coat (2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	1	Male	Microchip #0A01751807	Hair (plucked, stored in 2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	1	Male	Microchip #0A01751807	Plasma (2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	2	Male	Microchip #0A01751807	Protein Saver Card	Aug. 2 2019

Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	3	Male	Microchip #0A01750603	Blood (2mL tubes)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Male	Microchip #0A01750603	Buffy Coat (2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Male	Microchip #0A01750603	Hair (plucked, stored in 2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Male	Microchip #0A01750603	Plasma (2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	2	Male	Microchip #0A01750603	Protein Saver Card	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	5	Female	Microchip #0A01751302	Blood (2mL tubes)	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	Microchip #0A01751302	Buffy Coat (2mL tube)	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	Microchip #0A01751302	Hair (plucked, stored in 2mL tube)	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	Microchip #0A01751302	Plasma (2mL tube)	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	3	Female	Microchip #0A01751302	Protein Saver Card	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	4	Female	Microchip #0A01750920	Blood (2mL tubes)	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	Microchip #0A01750920	Buffy Coat (2mL tube)	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	Microchip #0A01750920	Hair (plucked, stored in 2mL tube)	Aug. 3 2019
Prolemur	Greater	Unknown	Wild	1	Female	Microchip	Plasma	Aug. 3

simus	Bamboo Lemur	(adult)				#0A01750920	(2mL tube)	2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	2	Female	Microchip #0A01750920	Protein Saver Card	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	4	Male	Microchip #0A01751109	Blood (2mL tubes)	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Male	Microchip #0A01751109	Buffy Coat (2mL tube)	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Male	Microchip #0A01751109	Hair (plucked, stored in 2mL tube)	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Male	Microchip #0A01751109	Plasma (2mL tube)	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	2	Male	Microchip #0A01751109	Protein Saver Card	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Male	Microchip #0A01752527	Blood (2mL tubes)	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	4	Male	Microchip #0A01752527	Buffy Coat (2mL tube)	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Male	Microchip #0A01752527	Hair (plucked, stored in 2mL tube)	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Male	Microchip #0A01752527	Plasma (2mL tube)	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	2	Male	Microchip #0A01752527	Protein Saver Card	Aug. 3 2019
Hapalemur aureus	Golden Bamboo Lemur	November 2016 (infant)	Wild	2	Female	None, preserved in freezer at Centre ValBio Madagascar	Tissue (2mL tube)	Found dead by local research technician,

								January 2016
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood in buffer (9mL tube: 3mL blood, 6mL buffer)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood in buffer (9mL tube: 3mL blood, 6mL buffer)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood in buffer (9mL tube: 3mL blood, 6mL buffer)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood in buffer (9mL tube: 3mL blood, 6mL buffer)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood in buffer (9mL tube: 3mL blood, 6mL buffer)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood in buffer (9mL tube: 3mL blood, 6mL buffer)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood in buffer (9mL tube: 3mL blood, 6mL buffer)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood in buffer (9mL tube: 3mL blood, 6mL buffer)	Planned January 2020
Hapalemur aureus	Golden Bamboo	Unknown (adult)	Wild	1	Unknown	Unknown	Blood in buffer (9mL	Planned January

	Lemur						tube: 3mL blood, 6mL buffer)	2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood in buffer (9mL tube: 3mL blood, 6mL buffer)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood in buffer (9mL tube: 3mL blood, 6mL buffer)	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood in buffer (9mL tube: 3mL blood, 6mL buffer)	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood in buffer (9mL tube: 3mL blood, 6mL buffer)	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood in buffer (9mL tube: 3mL blood, 6mL buffer)	Planned January 2020
Eulemur	Red-	Unknown	Wild	1	Unknow	Unknown	Blood in	Planned

rufifrons	fronted brown lemur	(adult)			n		buffer (9mL tube: 3mL blood, 6mL buffer)	January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020

6. The current location of the specimen(s):
Name: Dr. Patricia C. Wright
Address: 33 BP
City: Ranomafana
State/Province: Ifanadiana
Postal Code: 312
Country: Madagascar

7. Recipient:
Name: Dr. Patricia C. Wright
Address: N-203 Social and Behavioral Sciences Building, Stony Brook University
City: Stony Brook
State: New York
County, Postal Code: Suffolk, 11794-4310

SOURCE OF SPECIMEN

8. N/A

9. For **EACH** animal/specimen **taken from the wild**, provide the following:

a. Scientific name and common name: *Prolemur simus*, Greater Bamboo Lemur
Hapalemur aureus, Golden Bamboo Lemur
Eulemur rufifrons, Red-fronted Brown Lemur

b. Specific location of where, when, and by whom (name and address) the specimen was removed from the wild:

Species: *Prolemur simus*
Where: Karianga, Ivato, District Vondrozo, Madagascar
When: Aug. 1 – 3 2019. See table above for exact dates. Second sampling planned for January 2020 during translocation.
By whom: Dr. Patricia C. Wright, N-203 Social and Behavioral Sciences Building, Stony Brook University, Stony Brook NY 11794-4310, USA

Species: *Hapalemur aureus* and *Eulemur rufifrons*
Where: Ranomafana National Park, Districts Haute Matsiatra and Vatovavy-Fitovinany, Madagascar
When: January 2020. Collecting has not yet taken place.
By whom: Morgan Chaney, 750 Hilltop Drive, Room 226, Ken State University, Kent, Ohio 44242

c. Purpose of removal and length of time held in captivity:

Prolemur simus:
Purpose: Animals were captured to fit them with radio collars and be examined by a veterinarian prior to a translocation from a disturbed region to a protected region, Ranomafana National Park. They will be re-captured in January of 2020 for the translocation. Human disturbance in their current range negatively affects the lemurs' food supply and has in the past included illegal poaching. In their new range they will be both protected from human disturbance and have the potential to contribute to the genetic diversity of the severely declining population in the protected region. The radio collars will provide the means to track the animals when they are release in the National Park. While they were anesthetized, blood samples and measurements were also taken for the analyses described below, including understanding the population genetics of the species.
Length of time: Each animal was held for approximately 5-8 hours between time of darting and time of release. They were released after full recovery from the effects of the anesthesia.

Hapalemur aureus and Eulemur rufifrons:
Purpose: Animals are being darted and captured for the purpose of obtaining blood samples for the research described below in question 10 and in detail in the attachment "DDRIG Proposal_Chaney.docx" In short, the blood samples will be used to understand the genetics and biochemistry of cyanide adaptation. This species eats 12-50
Length of time: Each animal will be held for approximately 3-5 hours between time of darting and time of release. They will be released after full recovery from the effects of the anesthesia.

d. If and how any remuneration, either financial or in-kind, was provided for taking or capturing animals or for the collection of samples.

The supervising veterinarian, Dr. Hajanirina Rakotondrainibe, was and will be paid a standard rate for his services in evaluating and monitoring the health of the lemurs. Darting technician Velotsara Jean Baptiste was and will be paid a standard rate for his services in darting the animals.

No remuneration of any kind was or will be provided for the samples themselves.

e. Your efforts to use captive specimens, or parts thereof, in lieu of taking animals from the wild:

The *Prolemur simus* samples are from animals that are part of a translocation program to transfer animals from a disturbed region to a protected region, to protect these individuals and the future genetic diversity of the species. Currently, the only population in a National Park consists of two related individuals, a father and daughter. Thus, to understand the genetic consequences of this translocation, the samples must come from the individuals translocated, and their capture for fitting radio collars and health examinations provided the opportunity to collect high quality DNA samples without additional disturbance. High quality samples, such as from blood, are imperative because additional conservation genetic studies will be carried out including analyses of nutritional and disease adaptations in this population.

The *Hapalemur aureus* samples are from wild animals eating their natural diet. There are no *H. aureus* currently living in captivity anywhere in the world. Thus, to understand anything about their population genetics and adaptation, including inbreeding, population connectivity, and nutritional adaptation, requires sampling wild individuals in their native range. The *Eulemur rufifrons* samples will be used to compare the nutritional adaptations of a non-bamboo eating species (*E. rufifrons*) with the bamboo specialized and cyanide adapted *H. aureus*. It is necessary that samples are taken from individuals that live in the same habitat and are exposed to the same dietary options as the *H. aureus* individuals sampled, thus we cannot use samples from captive *Eulemur.*

f. Copies of your foreign or domestic collecting permit.

See attached **Autorisation de Recherche (Research Authorization)** from the Gestion des Ressources Naturelles Renouvelables et des Ecosystemes (Department for Management of Renewable Natural Resources and Ecosystems) in Madagascar. Original in French, translation provided. This permit applies to the *Prolemur simus* samples obtained in August 2019 listed in question 5.

A permit has not yet been granted for the additional *P. simus* samples that will be obtained during the second darting procedure immediately prior to translocation. Currently, the Malagasy government typically grants permits only once the research team is in Madagascar and ready to proceed with the project. In this case that will be January 2020. A permit application has not yet been submitted to the Gestion des Ressources Naturelles Renouvelables et des Ecosystemes for the *Hapalemur aureus* sampling project. Based on our previous successes obtaining permits for similar studies (including the included *P. simus* study) we anticipate receiving authorization for both of these sampling protocols. However if a permit is not granted, the *H. aureus* project will not go forward and no samples will be collected. If the additional *P. simus* sampling is

not approved, they will be transported (as previously approved in the Autorisation de Recherche) but no additional samples taken.

g. Documentation showing that the specimen(s) were legally obtained by the applicant.

See attached **Proces-Verbal de Constatation (Report of Observation)** from the Chef Cantonnement de l'Environment de l'Écologie et des Forêts (Station head for the Environment, Ecology, and Forests Department) in Vondrozo, Madagascar. Original in French, translation provided. This documentation is for the samples obtained during the first *Prolemur simus* darting, prior to translocation. In Madagascar, the Proces-Verbal de Constation is only completed upon completion of sampling, thus we do not yet have it for the second round of *P. simus* sampling (during the transportation part of the translocation project) or the *H. aureus* sampling. As this document is required to obtain a CITES export permit from Madagascar, neither the additional *P. simus* samples nor the *H. aureus* samples will be exported from Madagascar, and thus not imported to the United States, if it is not granted.

h. Copies of any applicable State, Tribal, Federal, or Foreign government permits or licenses that authorized the removal of this animal from the wild.

See attached **Research Authorization**, described above.

JUSTIFICATION FOR REQUESTED ACTIVITY.

10. Provide a detailed statement justifying the proposed activity, particularly the following:

a. Describe the purpose of your proposed activity.

Of a total world population estimated around 500-600 individuals, there are only two wild *Prolemur simus* individuals within a protected area. The rest are in fragmented populations subject to varying amounts of human disturbance. The two individuals living in a protected area, Ranomafana National Park (RNP) are a father and daughter, and despite the daughter having reached breeding age at least 5 years ago, they have not reproduced with each other nor have other *P. simus* individuals immigrated to expand the group. Thus the goal of the translocation project during which these samples were collected is twofold:

1. Increase the population size within RNP, hopefully increasing breeding opportunities and thus genetic diversity in a protected population;
2. Move a family group of *P. simus* individuals from a disturbed habitat in which they are currently at risk to the protected National Park.

The translocation occurs in two stages. First, the family group to be translocated has been identified, radio collared and examined, and the relevant samples taken. They were released at the darting site for monitoring until the translocation itself, when they will be re-captured and transported to Ranomafana National Park. Additional samples will be taken at this time.

The objectives are:

1. Translocate a family group from the unprotected region of Karianga to RNP.
    1. Fit captured individuals with radio collars to facilitate tracking once released,

2. Examine the health and parasite load of the individuals to be translocated, both pre- and post-translocation,
3. Monitor their health and behavior after capture in their native range,
4. Recapture and translocate to RNP

2. While anesthetized for the above procedures (#1/2 and #4), obtain blood (including buffy coat, plasma, and protein) and hair samples for further health, nutrition adaptation, and disease adaptation analyses.

The objectives of the *Hapalemur aureus* project are twofold:

1. Bamboo lemurs, including *H. aureus*, have some of the most specialized diets among all primates, and possibly the only primates genetically adapted to cope with high dietary cyanide (12-50 times the expected lethal dose). Blood samples from *H. aureus* and a related species with overlapping range, *Eulemur fulvus,* will be used to improve our understanding of their adaptation to this lethal toxin.
2. *H. aureus* is considered to be Critically Endangered by the IUCN, but currently nothing is known about their population genetics. These samples will also be used to determine the genetic health of the species, including testing for inbreeding, current and historical population size, and population fragmentation.

Four different types of analyses will be performed with blood samples:

1. Population demographic analyses to investigate the population history, heterozygosity, and potential inbreeding of both *P. simus* and *H. aureus*. Understanding these aspects of the population are important for continued conservation efforts. Population genetic analysis of *P. simus* has only previously been done using only five individuals from across the species' current range. This analysis will more than double the sample set, and allow greater depth of understanding of the population and inbreeding risk. Population genetic analysis of *H. aureus* has never been done.

2. Genomic adaptation analyses to investigate ecological correlates of disease adaptation in both *P. simus* and *H. aureus*. Wild lemurs are susceptible to many diseases, and this investigation will compare disease adaptations in populations that have been isolated for many generations, with different amounts of human contact (thus increased exposure to human and livestock diseases). In particular, both *P. simus* and *H. aureus* may have less exposure to vector-borne diseases than other lemur species, since their high cyanide diet may deter ectoparasites that spread disease. This analysis has not previously been carried out, and makes use of novel bioinformatic methods that require high quality assembled genomes with maximum contiguity of the genomic scaffolds. To this end, we will be conducting third-generation (long-read) whole genome sequencing, which requires high molecular weight DNA.

3. RNA expression analyses to investigate protein expression patterns associated with cyanide adaptation, which is unique among primates to bamboo lemurs. While previous work has identified genes involved with *P. simus* and *H. aureus* cyanide adaptation, the protein coding changes found are insufficient to entirely explain the extent of this unique adaptation. RNA expression analyses require blood samples, since the detoxification enzymes and cellular respiration associated proteins are expected to be expressed in the blood to combat circulating cyanide after it is

ingested by the lemurs. (Cyanide production is naturally used by the bamboo they eat as an anti-herbivory strategy.)

Because the translocation protocol involves capturing the animals twice (once for health assessment and radio collaring to facilitate monitoring before transportation, and once for the transportation itself), we will compare RNA expression in *P. simus* at two times of the year, once during the dry season when the animals are eating very little cyanide, and once during the wet season when the animals are eating a great deal of cyanide (the bamboo produces cyanide mainly in its actively growing structures). This will allow a seasonal comparison to validate that expression patterns are associated with dietary cyanide.

4. Chemical composition analyses of the blood to investigate concentrations of free cyanide and its primary metabolite, thiocyanate in both *P. simus* and *H. aureus*. These are expected in the blood based on previous non-invasive studies using urine, but their concentrations have never been directly measured. This will complement the previously-described RNA expression analyses, and requires blood samples. Likewise, we will compare chemical composition in *P. simus* at two times of the year.

The darting of lemurs is strictly regulated by Madagascar's Ministry of the Environment. Proposals for permits need to be submitted and approved before darting takes place. Centre ValBio's Institute for the Conservation of Tropical Environments ICTE representative in Madagascar's capital, Dr. Benjamin Andriamihaja, facilitates the submission of Dr. Patricia C. Wright's permit proposals. In order to dart animals, researchers must submit a proposal to Madagascar National Parks (MNP) describing why the darting is needed. The proposal for darting and translocation of *P. simus* was approved (please see attached Autorisation de Recherche and translation). The proposal for darting of *H. aureus* has not yet been submitted, but is expected to be approved. If it is not approved, the darting will not be carried out and no samples will be taken.

The process of darting will follow a standard methodology. Darting is performed by trained specialists, including Centre ValBio's affiliated veterinarian Dr. Hajanirina RAKOTONDRAINIBE (please see attached CVs). The darting team uses a CO2 air rifle, which launches light-weight 9-mm darts. The darts inject Telazol® at 10 mg/kg of body weight intramuscularly. Lemurs are caught individually in a large net held by four people as they fall.

Each anesthesized lemur is checked by the veterinarian (heart rate, lung listened to, body temperature taken), weighed and measured. Ectoparasites are removed for further investigation, the animals are checked for scars, nipples on the females are checked for milk, measurement of male testes is collected and other protocol specific measurements are taken. Blood is drawn and hair samples plucked. The data on each individual lemur is kept in a database at Centre ValBio. Each animal will receive a radio collar so that he/she can be followed. The ATM radio collars have been used previously on this species successfully. After the medical check-up and blood and hair sampling, the animals are allowed to recuperate from the Telazol in light-weight sacks (recuperation time ~ 3 hours) before they are released back to the darting site. Animals are followed and monitored for two to five hours to ensure complete recovery.

For further methodological details of darting, translocation, and sampling, please see the attached research proposals: "Updated_proposal_translocation_July19.docx" and "DDRIG Proposal_Chaney.docx".

b. Description of the technical expertise of each person as it relates to the proposed activities.

Dr. Patricia Wright, director of the Institute for Conservation of Tropical Environments, has been researching and protecting lemurs in Madagascar for 30 years, including helping to establish Ranomafana National Park to protect *Prolemur simus* and *Hapalemur aureus*. She has supervised more than two dozen lemur darting procedures in that time, including both of those species as well as other lemur species. Please see CV attached.

Dr. Hajanirina Rakotondrainibe is a wildlife veterinarian who has specialized in lemurs since 2007, and is a member of the Malagasy Order of Veterinary Doctors. He performed and will be performing the health monitoring and evaluation for all aspects of these projects. Please see CV attached.

Velotsara Jean Baptiste is a technician at Centre ValBio, Dr. Patricia Wright's research station, who specializes in lemur darting. He is the main darting technician at Centre ValBio and has experience safely darting *Prolemur simus*, *Hapalemur aureus,* and *Eulemur fulvus.*

Dr. Tobias Gräßle is a wildlife veterinarian who specializes in primate disease. He provided additional sampling supervision and health evaluation during the initial *Prolemur simus* capture. Please see CV attached.

Dr. Sina Feyer is a wildlife veterinarian who provided additional sampling supervision and health evaluation during the initial *Prolemur simus* capture. Please see CV attached.

Morgan Chaney is a PhD candidate in the Anthropology Department at Kent State University, where he is studying cyanide adaptation in *Hapalemur aureus* and *Prolemur simus*. Please see CV attached.

c. Copies of contracts, agreements or other documents that identify persons involved and dates of activities for which authorization is being requested.

Please see attached **Research Authorization**, described in 9f, that applies to samples obtained during the *Prolemur simus* translocation project. Please see attached "Chaney IACUC approved.pdf" that applies to samples that will be obtained for *Hapalemur aureus* nutrition and population genetics project.

11. A statement on how the activities will **enhance or benefit the wild population**:

Translocation:

The goal of the P. simus translocation project is to translocate a family group of P. simus from a threatened forest fragment to a protected national park where two individuals of the same species already reside. The translocation benefits the lemurs being translocated because they will go from a habitat where they are unwanted and threatened by agriculture and farmers (who have asked for their removal due to crop raiding) to pristine forest in a national park where they will be protected. This also benefits the two

lemurs within the park because they are a non-breeding pair who have no hope for producing offspring without the introduction of new Prolemur simus individuals. The blending of two unrelated family groups benefits Prolemur simus as a whole by increasing genetic diversity and therefore survivability of the species. The associated sampling will benefit the wild population in two ways, described below.

Understanding the population genetics of *Prolemur simus* and *Hapalemur aureus*: The collection of the *P. simus* samples is directly associated with the translocation project. The collection of both *P. simus* and *H. aureus* samples benefits their wild populations in three ways:

1. Understanding the population genetics of both species.
    a. *P. simus:* Population genetic analysis of *P. simus* has only previously been done using only five individuals from across the species' current range. This analysis will more than double the sample set, and allow greater depth of understanding of the population and inbreeding risk. Currently, levels of inbreeding are unknown in any of its populations. The historical population size of *P. simus* is estimated to be quite large, so current heterozygosity within the species may be higher than expected relative to its current population size. On the other hand, the current population is heavily fragmented, which may have resulted in each fragmented population being heavily inbred. This information about how inbred and how genetically fragmented the populations of this species are will impact future management decisions. The current translocation project is an urgently-needed stopgap, and the samples obtained during this process will improve future management plans.
    b. *H. aureus:* Population genetic analysis of *H. aureus* has never been done. As a result, nothing is known about their population genetics. Analyses of the genetic health of the species, including testing for inbreeding, current and historical population size, and population fragmentation, are urgently needed. Understanding these aspects of the population are important for continued conservation efforts. This information will impact future management decisions, including whether or not translocations or breeding programs are necessary for the health of the species.
2. Understanding of the unique nutritional needs of both species. The natural diet of both *P. simus* and *H. aureus* are heavily (90+%) dependent on woody bamboo, including *Cathariostachys madagascariensis*. Many woody bamboos in Madagsacar, including *C. madagascariensis*, produce cyanide as an anti-herbivory mechanism, thus these two species are the only known cyanide adapted primates.
    a. It is hypothesized that woody bamboo, particularly *C. madagascariensis,* provides a healthier diet for *P. simus* and *H. aureus* than other foods they may find in anthropogenically disturbed areas, such as rice and cassava. Not only do *P. simus* and *H. aureus* rely on specific species of bamboo, they may rely on bamboo stands growing under conditions that result in specific nutrient profiles. However, it is currently unknown if cyanide is an important aspect of a normal diet for these two species. For example, a high cyanide diet may deter ectoparasites, thus protecting them from some disease (see point 3, below).

b. By comparing the transcriptomes and blood chemistry of these two cyanide adapted species to those of a related species that shares their habitat (*Eulemur rufifrons*), both during a season of high cyanide ingestion and one of low cyanide ingestion, we can better understand how and why *P. simus* and *H. aureus* are so dependent on woody bamboo and, perhaps, cyanide. Because this reliance on woody bamboo may include stands growing under conditions that result in specific nutrient profiles, this research will improve current conservation efforts that include plans to propagate and re-establish stands of bamboo by improving our understanding of how and why their diets are so dependent on woody bambo.

3. Investigating ecological correlates of disease adaptation in both species. Wild lemurs are susceptible to many diseases. Some they are exposed to naturally, and some through increased exposure to humans and their livestock. In particular, both *P. simus* and *H. aureus* may have less exposure to vector-borne diseases than other lemur species, since their high cyanide diet may deter ectoparasites that spread disease. Understanding the extent of these adaptations, and thus their susceptibility, can inform conservation decisions with respect to controlling disease transmission to these endangered species.

Neither the sampling itself, nor the purposes of the research, will be detrimental to the survival of this species, nor will it be used for any commercial purposes.

All information gathered from this research, and its conservation implications, will be made available in published documents and research articles. We intend to publish this research in scientific journals, as well as make it available to the Madagascar National Parks and conservation organizations.

12. If live specimens are to be held in captivity as part of the proposed activity:
N/A, animals are not being maintained in captivity and were released immediately upon recovery from anaesthesia.

IMPORTS, EXPORTS, OR RE-EXPORTS

13. For shipment of LIVE specimens…
N/A, no live specimens are to be transported.

14. For import of live southern white rhinocerouses…
N/A, no rhinocerouses are involved in this work.

15. For import of LIVE CITES Appendix-1 listed marine mammal species…
N/A, no marine mammals are involved in this work.

16. For import of CITES **Appendix-1 listed species,** provide information to show the import is not for primarily commercial purposes:
Please see attached **Autorisation de Recherche (Research Authorization)** from the Gestion des Ressources Naturelles Renouvelables et des Ecosystemes (Department for Management of Renewable Natural Resources and Ecosystems) in Madagascar. Original in French, translation provided.

This research authorization shows that the imported samples will be used for conservation research, animal health evaluation, and basic research purposes only.

17. For export…

N/A, no samples will be exported from the United States of America.

18. If the specimen is being re-exported…

N/A, no samples will be re-exported from the United States of America.

Curriculum Vitae

Date of birth:

Nationality:

Education:

10/2010 – 03/2016

2007 – 2010

2000 – 2007

Practical Experience:

Since 10/2017

08/2016 – 09/2017

04/2015 – 06/2015

03/2015 – 04/2015

09/2014 –10/2014

03/2014 – 04/2014

04/2013 – 04/2014

10/2013

Additional skills

Language skills

Driving license

Diving certificate

[redacted]

Sina Feyer

Sina Feyer

[redacted]

Study of Veterinary Medicine, Justus-Liebig-Universität Giessen, Germany - Graduation with honors (final grade 1,85)

Training as a veterinary technician, small animal hospital "Tierklinik Lüneburg", Germany, (final grade „excellent")

High school "Christian-Gymnasium Hermannsburg", Germany

Veterinarian at the small animal clinic - section for exotic pets, zoo and wildlife, Department of Veterinary Medicine at the Freie Universität Berlin, Germany

Veterinarian at the small animal clinic "Kleintierspezialisten", Berlin, Germany

Veterinary externship at the Stuttgart zoo "Wilhelma", Germany

Externship at the Wildbase Hospital and at the Small Animal Hospital, Massey University, Palmerston North, New Zealand

Elective course (including lectures and practical activities) *"Animal protection of ornamental fowl and exotic wild animals"* at the Loro Parque, Tenerife, Spain and participation in the *"VIII International Parrot Convention"*

Externship at the clinic for small animals and exotics "Willenbockel & Völker", Burgdorf, Germany

Clinical part time traineeship at the Clinic for Birds, Reptiles, Amphibians and Fish of the Justus-Liebig-University Giessen, Germany

Elective course (including lectures and practical activities) *"Animal protection of ornamental fowl and exotic wild animals"* at the Loro Parque, Tenerife, Spain

Certification of competence for handling distance injection devices

[redacted]

Tobias Gräßle | [redacted]

CV Tobias Gräßle

Personal Details:

Date of birth: [redacted]

Nationality: [redacted]

Education and Civilian Service:

10/2010 – 04/2016	Study of Veterinary Medicine, Justus-Liebig-University Giessen, Germany Graduation grade: "excellent" (1,35)
10/2008 – 09/2010	Study of Biological Sciences, University of Konstanz, Germany
05/2007 – 01/2008	Civilian service, DLR Rheinpfalz (advisory scientific institute for agriculturalists), Germany
03/2007	Graduation from school with the Abitur (university entrance qualification), Hannah-Arendt Gymnasium Hassloch, Germany

Practical Experience / Work experience

Since 04/2019	Veterinary advisor for the "Hominoid Brain Connectomics Project", Max-Planck-Institute for Anthropology, Department of Primatology (Project Leader Catherine Crockford), Leipzig, Germany.
Since 09/2016	Veterinarian and PhD Candidate at Robert Koch-Institute (RKI), Berlin, Germany. Working group "Epidemiology of highly pathogenic Microorganisms" (Leader: Fabian Leendertz). Research focused on conservation, zoonoses and the One Health approach. Field missions to Dzanga-Sangha Protected Areas, Central African Republic and Taï National Park, Ivory Coast. Performing necropsies, wildlife anesthesia and sampling. Analyses of samples (on-site and at RKI) using methods like microbiology, serology and molecular biology (PCR, Sequencing).
18.05.2015 – 20.06.2015	Externship at the clinic of "The Raptor Center", University of Minnesota, St. Paul, USA Raptor rehabilitation, medicine and surgery, and captive raptor management
30.03.2015 – 15.06.2015	Externship at the veterinary department of the Frankfurt Zoo, Germany Zoo animal medicine and management
02.03.2015 – 27.03.2015	Externship at the avian and exotic section of the Small Animal Hospital, University of Queensland, Australia Medicine and surgery of native wildlife, birds, reptiles and small mammals

21.09.2014 – 03.10.2014	Repeated participation in the elective course "animal protection of ornamental fowl and exotic wild animals" at the Loro Parque, Tenerife, Spain. Combined with the attendance of the VIII international parrot convention.
17.03.2014 – 11.04.2014	Externship at the Mayfair Veterinary Clinic of James Harris, Tasmania,Australia Medicine and surgery of native wildlife, exotic pets and small animals
01.10.2013 – 11.10.2013	Participation in the elective course "animal protection of ornamental fowl and exotic wild animals" at the Loro Parque, Tenerife, Spain. Zoo animal management and medicine with an emphasis on birds.
01.10.2012 – 01.10.2013	Clinical part time traineeship at the Clinic for Birds, Reptiles, Amphibians and Fish, Justus-Liebig-University Giessen, Germany Emergency and weekend services, medicine and surgery of native wild birds, reptiles and exotic pets.
Winter term 2012/13 and Summer term 2013	Participation in the elective course: "wild ruminants"and in its framework the practical course "immobilization of wild animals". Lectures and hands-on training (assisting with the herd management of alpine ibex in a wildlife park). Acquiring the certificate to handle distance injection devices.
20.04 2013– 26.04.2013	Student assistant of the ©1st International Conference on Avian, Herpetological and Exotic Mammal Medicine 2013 (ICARE), Wiesbaden, Germany
25.01 2013– 27.01.2013	Excursion to the wildlife park „Dählhölzli" Bern, Switzerland. Practical endoscopy and ultrasound training in connection with the elective course „diseases of reptiles and amphibians"
Summer term 2012	Clinical part time traineeship at the internal medicine section of the Clinic for Small Animals, Justus-Liebig-University Giessen, Germany. Assisting during night emergency services
17.05 2010– 31.07.2010	Student research assistant, Max-Planck-Institiute for Ornithology, Radolfzell, Germany.

Honors:

- Award for excellent academic achievements given by the faculty of veterinary medicine of the Justus-Liebig-University Giessen.

Additional skills:

- Work and travel in Australia (03.02.2008 – 20.06.2008)
- Language skills: German (native tongue), English (fluent), French (medium level)
- Driving license
- Certification of competence for handling distance injection devices
- Diving license
- EDP knowledge: Microsoft-Office (Word, Excel, Powerpoint)

Dr Hajanirina Rakotondrainibe

DVM [redacted]

Tel: [redacted]

E-mail: [redacted]

Hajanirina.Rakotondrainibe@ambatovy.mg

EDUCATION AND DIPLOMA

November 2013 — Training

- Marking and care in Amphibian research: Field training at Mitsinjo breeding center, Andasibe, Tamatave

Training initiated by Chelter Zoo (England)

October– December 2009 — Internship

- Wildlife medicine and Lemurs Conservation at Duke Lemur Center (Duke University, North Carolina).
  - ➢ Certificate on Wildlife Medicine
- Visiting program at North Carolina States Veterinary School (NCS University, North Carolina).
  - ➢ Certificate for International Student exchange program
- Zoo Medicine and Wildlife Practice at Saint Louis Zoo (Saint Louis Zoo, Missouri)

Grant funded by Madagascar Fauna Group, Tamatave

July 2009 — Internship

- Zoo Medicine and Wildlife Practice at Johannesburg and Pretoria Zoo, South Africa

Grant funded by the comity of the First Symposium on Zoological Medicine

February– March 2009 — Visiting program

- Development of industries production: Poultry farm, Farming and milk production, product transformation, slaughterhouse,
- Development on pharmaceutics product, veterinary equipment and veterinary hospital,
- Affiliated veterinary profession in Zoo condition, drug and materials importation

Grant funded by Malagasy government for veterinary school visiting program in South Africa

June 2008 — Graduation (D.V.M degree)

Doctorate degree in Veterinary Medicine delivered by the Department of Science and Veterinary Medicine (D.E.S.M.V, Medicine Faculty, Antananarivo University)

- Thesis topic: Gastrointestinal parasites on introduced free ranging lemurs within Lemurs Park and evaluation of risk factors (Imerintsiatosika, Antananarivo).

September 2007 – February 2008 — Internship

Working in Laboratory environment

National Laboratory for Veterinary Diagnosis (L.N.D.V. Itaosy, Antananarivo).
Internship program includes: Parasites analysis, Serology, Bacteriology and Post mortem diagnosis.

February 2007 — **Training**

Grant funded by Durell trust Madagascar and IPS (International Primatology Society)

- Methods and techniques used in primatology research (Manombo, Farafangana).
  - ➢ Certificate on Primatology research

2001 – 2006 — **University**

Department of Science and Veterinary Medicine (DSMV);
Medicine Faculty, Antananarivo University

2001 — **Scientific Bachelor degree**

EMPLOYEMENT AND FIELD EXPERIENCES

2011 to 2014 — **Full time veterinarian**

Fauna responsible within environmental department at Ambatovy Mine site
Ambatovy minerals S.A., Moramanga, Tamatave
Task attributed concern: Lemurs biomedical survey, spatial monitoring, capture, necropsy and general veterinary practice on wildlife

August 2012 and November 2013 — **Veterinary consultant**

Consultant veterinarian for the gerp GERP ("Groupe d'Etude et de Recherche sur les Primates) for the translocation project "TSIBAHAKA" as part of crowned sifaka metapopulation management program

Mai 2007 to 2010 — **Half time veterinarian**

Zoological veterinary care at Lemurs Park
Imerintsiatosika, Antananarivo

July 2010 — **Field Assistant**

Research project on Neurobiology of Hibernation in *Cheirogaleus*: Neurophysiologic, Neuroendocrine, Metabolism and Genetic Mechanisms (Kirhindy , Morondava)

June– July 2009: — **Substitute Veterinarian**

Zoological veterinary care at Ivoloina Park
(Madagascar Fauna Group, Tamatave

July– October 2008, November– December 2008 / April - May 2009: — **Team leader**

Project under contract with Madagascar Biodiversity and Biogeography Project (M.B.B.P / Henry Doorly Zoo)
Research on lemur's biomedical survey as part of impact survey within Ambatovy mining

exploitation (Moramanga, Tamatave).

August 2007: **Field assistant**

Project under contract with Institute for the Conservation of Tropical Environments (I.C.T.E.) and Saint Louis Zoo
Research on lemurs' biomedical survey
Betampona special reserve, Tamatave

March to May 2007: **Field assistant**

Project under contract with Institute for the Conservation of Tropical Environments (I.C.T.E.)
Research on Census and ecology of *Eulemur Albifrons*
Betampona reserve, Tamatave

SCIENTIFIC MEETING ATTEMPTED

Octobre 2014

Workshop on zoo management and veterinary care
Ivoloina park, Tamatave
Initiated by Forest and Water Ministry of Madagascar with participation of zoos collectives
Speech topic: Importance of veterinary care in Zoo breeding

February 2014

Workshop on chytride detection and disease control for Amphibians
DGF Antananarivo
Initiated by Forest and Water ministry of Madagascar with participation of zoos collectives

August 2013

5th International Prosimian Congress
Centre ValBio, Ranomafana, Madagascar

July 2012

IUCN redlist review and long term conservation program for Lemurs of Madagascar
Carlton, Atntananarivo

Mars 2012

Festival "Lemurs day"
Museum of Natural History, Paris, France

Mars 2012

Prosimian TAG meeting
European Assosiation of Zoo and Aquarium « EAZA », Rheine, Germany

November 2011

« Colloque Conjoint Parasitologie-Célébration Vet 2011 »
Malagasy Academie of Science, Antananarivo
Speech topic: Ambatovy conservation approaches through lemur's biomedical survey: Methods, preliminary results and research perspectives

July 2009

First Symposium on Zoological Medicine
Johannesburg, South Africa
Speech topics: Zoo Practice and Challenge in Madagascar

May 2009

Workshop on Zoo and Conservation Medicine
Zoo Conservation and Medecine Club", Ivoloina Park, Tamatave
Speech on Zoo practice at Lemurs Park

PUBLICATIONS

- « Nedbank Capital Sustainable Business Award » for Ambatovy project through the development of sustainable biodiversity conservation program http://www.capital.nedbank.co.za/capital/press-room/2014-nedbank-capital-sustainable-business-awards-r
- Sifaka conservation pour la sauvegarde des Propithecus couronnés http://www.sifaka-conservation.org/2014/10/23/translocation-reussie/
- Les approches du projet Ambatovy sur la surveillance de l'état de santé des lémuriens dans leur site minier: approches méthodologiques, résultats préliminaires et perspectives http://www.recherches.gov.mg/IMG/pdf/com42A52.pdf
- The Relationship of Sleep with Temperature and Metabolic Rate in a Hibernating Primate. *PLoS ONE*, 2013; 8 (9): e69914 DOI: 10.1371/journal.pone.0069914
- Documentary on wild animals translocation: "66 minutes grand format" https://www.youtube.com/watch?v=ydMCYJUwu-w

LANGUAGE AND SKILLS

[redacted]

Computer skills: Microsoft office (worlds, excel, power point ....) and basic software
Others: Radio telemetry, G.P.S .....
Driving license: B Category

MEMBERSHIP

O.N.D.V.M: National Order of Malagasy Veterinarian Doctor
G.E.R.P: Study Group and Research in Primate
Z.C.M.C: Zoological and Conservation Medicine club

Doctoral Dissertation Research: Investigating Genomic and Expression-level Adaptations for Cyanide Detoxification in *Hapalemur aureus*

I. Introduction

Bamboo lemurs have some of the most specialized diets among all primates, focusing their feeding on a small number of grass or sedge species in all habitats where they have been studied (Table 1; refs. 1–4), but the niche inhabited by these animals is not only one of dietary specialization. The three species in and around Ranomafana National Park are known to focus most, or nearly all, of their feeding time on various parts of Malagasy giant bamboo (*Cathariostachys madagascariensis*), which exposes two of these species (*Hapalemur aureus* and *Prolemur simus*) to lethal levels of cyanide (CN^-) on a daily basis (5). **Because of their exposure to this poison, these lemurs defy current understandings of cyanide toxicology, ingesting daily 12-50 times their estimated lethal dose** (5–7). It can be inferred that the lemurs are absorbing cyanide into the bloodstream (8), and they are excreting it in both its original form and in the detoxified form of thiocyanate (9). However, despite the three decades that have passed since the discovery of this unique feeding strategy (5), researchers still have no compelling evidence to explain how bamboo lemurs cope with cyanide and ostensibly avoid its toxic effects.

The severity and diversity of cyanide's effects illustrate the strength of bamboo lemurs' adaptation to tolerate this poison. The toxicity of cyanide stems from its ability to inhibit aerobic respiration by inactivating a critical portion of the electron transport chain, specifically the second subunit of cytochrome c oxidase (10). In doses exceeding only a few milligrams CN^- per kilogram body weight (see Table 2), this mechanism targets the most energetically active tissues, especially the brain and heart (11, 12). Prior to death, symptoms of acute cyanide exposure escalate from dizziness, nausea, and tachycardia to hyperventilation, seizures, and coma (10, 13). If exposed to lower doses over a long period, an animal can be expected to present the symptoms of chronic cyanide intoxication, attributable to both cyanide as well as its metabolite thiocyanate (14). Two primary symptoms accompany chronic cyanide exposure: disruptions to thyroid-hormone synthesis, leading to hypertrophy of the thyroid itself (15–17); and signaling defects to descending motor neurons (18, 19), leading to ambulatory difficulties or ataxia (20, 21). In this light, the estimated daily dose of cyanide for *Hapalemur aureus*, approximately 190 mg CN^-/kg body weight (calculated from ref. 6), is simultaneously impressive and puzzling. How are these lemurs able to avoid such broad and drastic effects?

We will test the hypothesis that *H. aureus* is genetically adapted to cope with cyanide through a heightened ability to detoxify this potent toxin. This will entail the use of genomic, transcriptomic, and metabolomic methods to accomplish the following three objectives. **First,** we will test for positive selection in genes in metabolic pathways known to be linked to cyanide detoxification. **Second,** we will examine whether there is evidence of adaptive changes at the level of gene expression in *H. aureus* by comparing the whole-blood transcriptomes of free-ranging animals against those of captive *L. catta*. **Third,** we will evaluate whether the chemical composition of *H. aureus* blood serum comprises increased levels of the principal substrate for cyanide detoxification, thiosulfate. In total, this dataset will allow for powerful inferences into the adaptive components of *H. aureus* blood, a tissue that is of special interest because of its upstream position in the toxicological cascade initiated by cyanide.

II. Theoretical Background

The initial discovery of *Hapalemur aureus* (22) occurred during the beginning phases of the establishment of Madagascar's Ranomafana National Park, and this new species helped to justify the very foundation of the park (23). Also during this period, Glander et al. (5) made the surprising discovery that *H. aureus* and the related species *Prolemur simus* were consuming levels of cyanide that were at least 12 times higher than those that would kill other animals of equivalent weight. Since then, much has been learned about the diets of bamboo lemurs. Additionally, the toxicology literature is rich with information about the symptoms of cyanide poisoning, their physiological mechanisms, and mechanisms for detoxifying this poison. In this section, we will briefly review these two bodies of literature and ultimately argue that our research hypotheses would be best addressed through "omic" methods.

Dietary Specialization of Bamboo Lemurs

The typical diet of bamboo lemurs is among the most specialized of all primates. Despite their diminutive body sizes (0.9-2.6 kg: ref. 24), these lemurs inhabit a folivorous niche that is often compared with that of the giant panda (24–26); and they are the only extant primates known to specialize so intensely on bamboo or grass species (2, 24, 27). All three species at Ranomafana National Park (i.e., *H. aureus, H. griseus*, and *Prolemur simus*) consume diets that are mostly composed of a small number of species of bamboo or liana (3, 4, 28). *P. simus* is the most specialized feeder, concentrating nearly all of its feeding on the pith or shoots of Malagasy giant bamboo (*Cathariostachys madagascariensis*) in 95% of feeding records across more than one year of continual data collection (4, 25). In contrast, *H. griseus* shows the least reliance on bamboo or lianas, although feeding records for this species are still extremely biased toward bamboo (55%-88% of feeding records: refs. 3, 4, 28). *H. aureus* lives on a diet that appears at first to be very similar in diversity to that of sympatric *H. griseus* populations (4), but the two species are distinguished by their differential reliance on separate plant parts from the same species of giant bamboo (5). The specific parts consumed by *H. aureus* exhibit more antifeedant compounds, in the form of cyanogenic glycosides, than those consumed by *H. griseus* (5, 6). Although other species outside of Ranomafana (e.g., *H. meridionalis, H. alaotrensis*) do not actually consume bamboo, they nonetheless eat grasses (Poaceae) or sedges (Cyperaceae) at similarly high frequencies (Table 1; refs. 1, 29). Thus, regardless of habitat or species, bamboo lemurs subsist on constrained, highly folivorous diets.

Because of the specialized diets of bamboo lemurs, the evolutionary trajectory leading from their common ancestor with *Lemur catta* represents a departure from the norms of primate biology, wherein dietary generalization with a focus on frugivory is a constant theme; nonetheless, some familiar patterns are evident. Like other folivorous primates (e.g., *Alouatta* spp.: refs. 30, 31, Colobini: ref. 32), bamboo lemurs exhibit energy-minimizing activity patterns that heavily emphasize resting and feeding, which respectively compose 41%-54% and 37%-48% of total activity budgets from the three Ranomafana species (24). Aside from this typical behavioral accommodation to folivory, reliance on such a fibrous diet has produced anatomical adaptations that are mirrored by other folivorous primates. The gastrointestinal (GI) tract of *H. griseus* and *P. simus* differ from other lemuroid species (33–36). In their description of the GI tracts of five species of lemur, Campbell et al. (35) showed that *H. griseus* departs from other lemur species in having a haustrated colon and a relatively blunt and abbreviated cecum. Haustration of the portion of the intestine aborad to the cecum is generally seen as an accommodation to hindgut fermentation of the complex polysaccharides found in foliage (37, 38); this form of fermentative digestion is common among folivorous primates (38–41) and is apparently exhibited by bamboo lemurs based on their GI anatomy (34–36). Of these two species, *H. griseus* showed further support for the expectation that they are hindgut fermenters in their much longer mean transit time (i.e., 18.21 hours) than those of other lemurid species (35, 42). The peculiarity of the diet of these lemurs is therefore reflected in their GI anatomy and physiology in ways that would be expected based on primatological theory; however, one aspect of bamboo-lemur dietary ecology has eluded explanation for several decades.

The most surprising component of the diet of these animals is the inclusion of cyanide levels far above their estimated lethal dose (5, 6) and at levels that are currently unparalleled among mammals (see ref. 43). Thirty years ago, Glander et al. (5) measured the cyanide content of various parts of two species of bamboo that were known to be consumed differentially among the three species of bamboo lemur at Ranomafana, and they inferred that *H. aureus* consumed 12 times the lethal dose for a mammal of their body size. More recently, Ballhorn et al. (6) used a more sensitive spectrophotometric method to quantify the cyanogenic potential of major plant parts for the principal bamboo species consumed by these lemurs (*Cathariostachys madagascariensis*), as well as for different ontogenetic phases within these plant parts. These measurements proved to be approximately four times those taken by Glander et al. (1989), indicating an even higher level of daily cyanide (CN^-) exposure for *H. aureus*, estimated at about 190 mg CN^-/kg body weight (6)! Because *P. simus* is known to devote about 30% more time than *H. aureus* to feeding on this species of bamboo (4), its intake levels should approximate those of *H. aureus*—especially in light of the former's proclivity for branch and ground shoots (4), the most cyanogenic parts of *Ca. madagascariensis* (6). The third species of bamboo lemur at Ranomafana is *H. griseus*, whose diet

is more varied than those of its sympatric relatives (3, 4). Nonetheless, *H. griseus* consumes cyanogenic parts of *Ca. madagascariensis*, but at lower levels compared to *H. aureus* and *P. simus* (4, 8, 24). The rates of cyanide ingestion for these two latter species become especially interesting when compared to similar data from perhaps the most famous bamboo specialist among mammals, the giant panda (43). In a month-long feeding trial in which 20 giant pandas were fed a naturalistic diet of Chinese bamboo (*Chimonobambusa szechnuanensis*), Huang et al. (43) estimated that the daily cyanide consumption for these animals was only about 0.52-0.56 mg CN^-/kg body weight. To our knowledge, no comparable data on cyanide exposure exist for any other bamboo-specializing mammal (e.g., *Ailurus fulgens*); therefore, we provisionally conclude that ***H. aureus* and *P. simus* are superlative in their ability to tolerate higher levels of cyanide than any other mammal currently known.**

Cyanide Intoxication and Detoxification

Cyanide is a poison dreaded for its ubiquity among plants (44, 45), low threshold for toxicity (10, 46), and history as a chemical warfare agent (47, 48). At high levels of exposure, acute cyanide toxicity is primarily defined by the inhibition of cytochrome c oxidase (COX), the final complex in the electron transport chain (10, 46, 49), especially in neural tissue (50). Long-term cyanide exposure presents diverse indicators (14, 47, 51), including endocrinological (15, 16) and neurological symptoms (18, 50, 52). Regardless of exposure time, cyanide also inhibits many metalloproteins by forming ionic bonds with the metallic cations that are so crucial to the functions of these proteins (46, 53) and depletes levels of important and rare nutrients such as selenium and vitamin B_{12} (54), presumably by a similar chemical mechanism. Therefore, cyanide should be viewed as a general metabolic inhibitor (55, 56) because of its broadly toxic effects on the body.

Inhibiting the final complex of the electron-transport chain is harmful for two reasons. First, COX inhibition causes cells to rely on anaerobic respiration (10, 46), a pathway that lowers the synthesis of ATP by about 95% (57, 58) and leads to a decrease of pH in the cell because of the buildup of metabolites such as lactic acid (59). Second, COX inhibition also triggers cellular events that can lead to cell death, such as reduction of the mitochondrial membrane potential (60), heightened levels of radical oxygen species (ROS) (58), and increased cytosolic Ca^{2+} (57). Up to this point, this cascade would be deleterious for all cell types containing mitochondria, but experimental work using cultured neurons has shown that neurons are vulnerable to additional toxicity.

The primary target organ of cyanide intoxication is regarded to be the brain because of this organ's sensitivity to O_2 deficits (50, 61). Additionally, upper motor neurons appear to be especially vulnerable to cyanide-induced toxicity compared to other neuronal cell types (18, 50). This susceptibility may be due to the fact that this type of neuron uses glutamate as a neurotransmitter (62). Cyanide has been shown in cell-culture experiments to modulate glutamatergic NMDA receptors, which transmit Ca^{2+} ions upon activation (63, 64). Experimental work using various types of cultured neurons has demonstrated that cyanide exposure causes calcium ions to flood into the cytosol from multiple sources, not simply through NMDA receptors (e.g., voltage-gated Ca^{2+} channels: ref. 65). Compounding the resultant influx of extracellular calcium are additional releases of Ca^{2+} from the mitochondrial matrix (50) and endoplasmic reticulum (66)—both consequences of the increase in ROS production mentioned above (67, 68). Such rises in cytosolic calcium, as well as inhibition of COX, have been shown to initiate apoptotic or necrotic signaling cascades in neurons depending on neuronal cell type (60, 67, 69–72). In light of these mechanisms, an explanation begins to emerge for the neurodegeneration observed in animal models of long-term cyanide exposure (17, 51, 73), and the same can be said regarding the characteristically "Parkinsonian" presentation of acute cyanide toxicity (13) because the central etiology for Parkinson's disease involves glutamatergic dysfunction (74).

Chronic cyanide exposure is commonly observed in humans living in parts of Africa where the cyanogenic tuber cassava (*Manihot esculenta*) is a staple food (18, 20, 21). Chronic exposure is also associated with two similar neurological disorders: spastic paraparesis (or *konzo*) and tropical ataxic neuropathy (TAN), both of which involve dysfunction of motor neurons (14). *Konzo* is characterized by the abrupt onset of muscular stiffness or paralysis and damage to upper motor neurons (18). Similarly,

TAN is defined by paresthesia or ataxia in the lower extremity as well as blurry vision (20). These conditions have been correlated with serum thiocyanate levels as well as local importance of cassava as a staple crop in Nigeria (20) and the Democratic Republic of Congo (19). Researchers have also noted that the low levels of sulfur-containing amino acids (e.g., cysteine, methionine) present in cassava may make these populations vulnerable to such neurological conditions because of an already depleted pool of substrate for the primary pathway by which cyanide is detoxified (see below; refs. 19, 75, 76).

Aside from neurological pathologies (14, 50), chronic cyanide exposure can cause hypothyroidism through competition between thiocyanate and iodide for uptake by thyrocytes (16, 77). Because iodide is a critical component of thyroid hormone, this competitive inhibition leads to a depression in thyroid-hormone synthesis and causes hyperplasia of the thyroid, resulting in a goiter (16, 54, 78). Thiocyanate has also been suggested to directly inhibit the later stages of thyroid-hormone synthesis by interacting with catalytic sites on the enzyme thyroid peroxidase (15, 79). Notably, this endocrinological disruption is caused by thiocyanate, which is regarded as the product of cyanide detoxification. For this reason, the term *detoxification* is something of a misnomer: cyanide is seldom rendered fully harmless, even after transformation to thiocyanate, unless it is eliminated from the body.

Mammals are able detoxify cyanide at low doses by a variety of conserved mechanisms, but foremost among these is a pathway that uses sulfurtransferase enzymes, which detoxify a large majority of the cyanide in the bloodstream (80–82). Aside from this primary mechanism, a number of minor pathways for cyanide detoxification have been reviewed in the literature, acting on small proportions of the total cyanide: the non-enzymatic conversion of cyanide to 2-aminothiazoline-4-carboxylic acid in the presence of cystine (57, 81, 83); biotransformation into non-toxic one-carbon molecules, including exhaled CO_2 (84, 85); formation of protein-cyanide adducts (86, 87); and formation of covalent bonds with metallic ions of non-protein biochemicals (e.g., vitamin B_{12}, or cobalamin) (88, 89).

The enzymatic detoxification of cyanide is accomplished using the closely related enzymes known as thiosulfate sulfurtransferase (TST, or rhodanese) and 3-mercaptopyruvate sulfurtransferase (MPST). Both of these enzymes work by enzymatically transforming cyanide (CN^-) into thiocyanate (SCN^-), which is much less toxic than cyanide (55, 90). The source for the critical sulfur atoms needed for this process is sulfane sulfur, which is ultimately derived from cysteine (91, 92). Recent experimental work suggests that detoxification levels can be augmented by inducing more rhodanese expression and, importantly, by adding sulfane to the culture (55, 56). For example, Cipollone et al. (56) induced increased expression levels of bacterial rhodanese in engineered *E. coli*, and demonstrated that these cultures displayed increased viability compared to non-induced controls. The inference of increased expression of rhodanese complements recent immunohistochemical results from the livers of the giant panda (see above), which showed that these bamboo specialists express more rhodanese than *Felis catus,* a carnivoran mammal that consumes no bamboo (43). The effect documented by Cipollone et al. was significantly improved by the addition of extra thiosulfate to the medium, leading them to conclude that the transformation of cyanide to thiocyanate is limited by rhodanese's access to its substrate (56). Thus, cyanide can be detoxified to thiocyanate, and this reaction can be modulated by expression of sulfurtransferase enzymes and by the availability of sulfane on which these enzymes act.

In summary, the multifaceted molecular interplay initiated by cyanide involves disparate cellular systems, but protective detoxification can occur if cyanide is present below certain thresholds, which are likely determined by levels of sulfurtransferase expression (e.g., rhodanese) and sulfane sulfur (e.g., thiosulfate). If present above these thresholds, cyanide drastically reduces aerobic cellular respiration and initiates cell death, especially in neurons. Sublethal levels of cyanide over prolonged periods cause a second set of symptoms that are related to the metabolism of thyroid hormone. Clinically, acute and chronic cyanide exposure elicit symptoms that are not easily overlooked. These well-characterized mechanisms underscore the inference that bamboo lemurs (especially *Hapalemur aureus* and *Prolemur simus*), which apparently do not present these symptoms, must have undergone strong natural selection since their divergence from the lineages of *Lemur catta.* Because the effects of acute and chronic cyanide intoxication are so broad, the powerful and hypothesis-generating tools offered by the burgeoning fields

of genomics, transcriptomics, and metabolomics are especially well-suited for the evolutionary question of bamboo lemurs' exemplary cyanide tolerance.

III. Hypotheses

Because of cyanide's inferred presence in the bloodstream of *H. aureus*, we propose the general hypothesis that bamboo lemurs, and *H. aureus* in particular, are genetically adapted to detoxify cyanide at a higher rate than *Lemur catta*, a confamilial dietary generalist (93, 94). We will test two non-exclusive genomic hypotheses about their presumed adaptation to tolerate this poison:

Hypothesis A: *Hapalemur aureus* has adapted to detoxify its highly cyanogenic diet through positive selection on genes involved in the metabolism of sulfur or sulfur-containing amino acids (e.g., cysteine, methionine).

Prediction A1: Signatures of positive selection will be observed for the CDS of such genes in a phylogenetic comparison of *H. aureus, H. griseus, P. simus*, and *L. catta*. These signals of selection will be observed by estimating ratios of nonsynonymous to synonymous codon substitutions (*dN/dS*, or ω; ref. 95) on protein-coding DNA, and we specifically predict that *H. aureus,* or the bamboo-lemur last common ancestor (BL-LCA), will show estimates of $\omega > 1.0$ for genes involved in sulfur metabolism. We expect this outcome because selection on these genes would modulate the available sulfane that sulfurtransferase enzymes require in order to transform CN^- to SCN^- (55, 91, 96). Such changes would hypothetically be linked to an increased ability to detoxify cyanide through action by sulfurtransferases such as rhodanese or MPST.

Prediction A2: Positive selection will be inferred for *H. aureus* or the BL-LCA in non-coding *promoter* sequences of these same genes in a phylogenetic test involving the same taxa. This will be tested with a metric developed by Wong and Nielsen (97), a ratio known as ζ (pronounced *zeta*), which is conceptually similar to ω but is designed for non-coding DNA. We predict estimates of $\zeta > 1.0$ for *H. aureus* or the BL-LCA as compared to *L. catta*. This outcome would be interpreted as selection on the *regulation* of these pathways instead of, or perhaps in addition to, selection on the CDS of genes therein.

Hypothesis B: *Hapalemur aureus* detoxifies its high levels of circulating cyanide through adaptive changes to the composition of its bloodstream.

Prediction B1: A subset of cyanide-protective genes will be up-regulated in the blood of *H. aureus* relative to the most closely related species of dietary generalist, *L. catta*. To test this prediction, we will use RNA sequencing (RNA-seq) to characterize the gene-expression profile in the blood of *H. aureus*. These data will be combined with pre-existing RNA-seq data from *L. catta* through the publicly available Nonhuman Primate Reference Transcriptome Resource (98, 99). We expect these changes because, following absorption by the GI tract, the bloodstream is cyanide's courier to the rest of the body and should therefore harbor important information about the cyanide adaptation of these animals.

Prediction B2: The serum fractions of blood from *H. aureus* will show increased relative quantities of cyanide, thiocyanate, and thiosulfate. Demonstrating heightened levels of the first two molecules will be empirical confirmation of their inferred presence based on urinary data (9, 100). The last of these, thiosulfate, is regarded as rhodanese's canonical substrate for the enzymatic conversion of CN^- to SCN^- (see KEGG reaction R01931), a fact that is evident in one of rhodanese's synonyms, thiosulfate:cyanide sulfurtransferase. Increased levels of serum thiosulfate would be hypothetically linked with greater substrate availability for rhodanese to use in the detoxification reaction and would be consistent with the hypothesis of Cipollone et al. (55), that the enzymatic detoxification of cyanide is limited by the availability of sulfur. As with the previous prediction, this prediction will be tested through a pairwise comparison between *H. aureus* and *L. catta*.

Though we are testing a primary hypothesis of *genetic* adaptation to cyanide, two non-genetic alternatives bear some mention as well. One alternative is that bamboo lemurs are preventing the effects of cyanide through some supplement to the diet that would adsorb the compound prior to its uptake in the

intestine. For example, red colobus monkeys (*Piliocolobus kirkii*) consume the cycad plant *Encephalartos hildebrandtii*, which is known to contain nontrivial amounts of cyanide as well as high levels of toxic phenolic compounds (101, 102). These monkeys appear to be solving this problem by consuming charcoal, a substance known to experimentally lower cyanide mortality (103), immediately before ingesting their cycad meals (102, 104, 105). Similarly, the consumption of clay by psittacid parrots in Peru has been claimed as a mechanism to inactivate toxic alkaloids in their diet (106). A second alternative hypothesis concerns a possibly adaptive function of the bamboo-lemur microbiome. Recently, the gut microbiome of *H. griseus* has been shown to be convergent with that of the giant panda as compared to two phylogenetic controls, and this similarity was attributed to their common reliance on bamboo as a primary food (26). Therefore, it may be suggested that some portion of this derived microbial profile could be used to detoxify the cyanide in the bamboo-lemur diet, as various microbes have demonstrated abilities to inactivate toxic materials (e.g., refs. (107, 108)) including cyanide (55, 56).

We regard both of these possibilities as unlikely for two main reasons. First, although the three Ranomafana species of bamboo lemur have been observed to consume soil (4), this food was rarely eaten, composing less than 1.5-5.0% of their annual diet; moreover, the lowest levels of consumption were observed for *Prolemur simus*, the species that most highly specializes on Malagasy giant bamboo (4). Second, if the adsorption hypothesis were true *or* if cyanide were detoxified or sequestered by the gut microbiome, then the incidence of the poison would be highest in the feces and negligible in the urine because the cyanide would be kept out of the bloodstream (103). To the contrary, data from Yamashita et al. (8) regarding the urine and feces of *Pr. simus* and *Hapalemur aureus* show just the opposite pattern. Therefore, we regard it as unlikely that bamboo lemurs are avoiding cyanide's toxic effects through either supplemental additions to the diet or adaptive components of the gut microbiome.

IV. Materials and Methods

Aside from the materials we have already collected from the Duke Lemur Center (see below), the co-PI will collect blood and tissue samples from free-ranging *Hapalemur aureus* in Ranomafana National Park in Madagascar. For the generation of the high-quality genome assembly, we will retrieve samples from a deceased immature *H. aureus* that is currently in ultracold storage at Centre ValBio, the field research station at Ranomafana; this animal was collected by our collaborator M. Elise Lauterbur during a previous field season. Fresh blood samples, two tubes of $\geq$ 2 mL from each animal, will be collected from living animals that will have been sedated for this purpose. Though invasive, this procedure will be carried out by an experienced marksman and under the supervision of a licensed veterinarian and we will not remove the animals from their habitat during the protocol. All blood samples will be immediately transported back to Centre ValBio. Here, we will immediately store the samples to be used for later RNA extraction at -80°C, while the samples to be analyzed by GC/MS will be centrifuged in order to pellet the hematocrit fraction and isolate the blood serum. From these pelleted samples, serum will be transferred to a separate tube and stored at -80°C. The samples will be transported back to Kent State University using a vapor-phase nitrogen shipper, after which we will use the methods outlined below to test our hypothesis at three biological levels: the genome, the whole-blood transcriptome, and the blood-serum metabolome.

Genomic Data

We will extract high molecular-weight genomic DNA (HMW gDNA) from the existing specimen of *H. aureus*, currently in ultracold storage at Centre ValBio, and ship to the NYU Genomics Core for sequencing using the 10X Genomics Chromium system. This sequencing system allows the cost-effective retrieval of long-range information despite using a standard short-read Illumina sequencing platform (see ref. 109 for detailed explanation). Briefly, this information retrieval is accomplished by partitioning and sequencing barcoded linked-read libraries constructed from HMW gDNA (length per molecule $\geq$ 50 kb). The linked reads are constructed by loading as few as five molecules of HMW gDNA onto individual gel beads in an oil emulsion, thus creating so-called gelbeads-in-emulsion (GEMs, ref. (109)). Each GEM contains many copies of a single barcode out of one million distinct such oligonucleotides. After these GEMs are incubated, the result is many barcoded gDNA fragments that are suitable for high-throughput

short-read sequencing; and information from the one million separate barcodes can be used to identify fragments that came from the same molecules of HMW gDNA (109). These high-throughput sequencing data can then be used to construct de novo assemblies of about 60x coverage using the Supernova assembly algorithm (Table 3; ref. 109).

Using assembly gap-filling softwares (e.g., RAILS and Cobbler: ref. 110), we will then combine the Supernova-assembled *H. aureus* genome with a forthcoming de novo genome assembly from the same animal; the goal for this stage will be a larger, more contiguous genome assembly. This will be possible because, at the time of writing, we and our collaborators at Stony Brook University (M. Elise Lauterbur, Dr. Liliana Dávalos-Álvarez, Dr. Patricia C. Wright) have submitted *H. aureus* gDNA to NYU for one lane of 150-bp paired end sequencing on an Illumina HiSeq 4000; this sample, which is currently awaiting sequencing, is from the same specimen that we will use for the 10X Chromium genome described immediately above. **This will yield a highly contiguous, reference-quality genome assembly for *H. aureus* with a coverage of about 60x.** In the two most recent cases where mammalian genomes were assembled following this workflow (111, 112), researchers were able to identify complete copies of over 90% of the highly conserved Benchmarking Universal Single-Copy Orthologs (BUSCOs) for that eukaryotic taxon (113).

For the *Lemur catta* genome, we will use an Illumina HiSeq 4000 to sequence the genome of a male animal from the Duke Lemur Center, for which we already have fresh blood samples in ultracold storage. We will use one lane on this platform for 150-bp paired-end sequencing, which should yield an estimated coverage of approximately 30x (114). After trimming the FASTQ reads, we will assemble these reads using a reference-guided de novo approach with any or all of three related lemurid genome assemblies as references: the *H. aureus* assembly outlined above, our laboratory's existing genomic assembly for *H. griseus* (Table 3; see Section V) and the recently published assembly for *Prolemur simus* (115). Reference-guided methods have been shown to improve the contiguity and overall quality of eukaryotic genome assemblies (116, 117).

Following others who have used 10X Chromium sequencing (111, 112, 118), we will automate the annotation of our assemblies with MAKER (119). This program masks low-complexity regions with RepeatMasker (120) and then proceeds to use evidence from several independent sources to generate consensus gene models. The sources of this evidence are *ab initio* gene-prediction programs such as Augustus (121), SNAP (122), and GeneMark-ES (123), all of which can optionally be integrated with information from mapping RNA-seq reads to the genome using tBLASTn and BLASTx (119), which we will have for *H. aureus* and *L. catta* through the gathering of whole-blood transcriptomic data (see below). This will allow us to build a database of annotations that we can use to test Hypothesis A.

From this database of annotated genes, we will be able to locate and extract sequences (i.e., CDS, intronic DNA, 2 kb upstream from TSS) for genes related to the metabolism of sulfur (e.g., KEGG pathway hsa00270) from the genomes of *H. aureus, H. griseus, P. simus,* and *L. catta*. The source for information about metabolic pathways will be empirical databases such as those maintained by the Gene Ontology (GO) Consortium (124, 125) or the Kyoto Encyclopedia of Genes and Genomes (KEGG) (126). Following extraction of these sequences, we will estimate the *dN/dS* ratios of these genes and model their ancestral states using software packages such PAML (127) and/or HyPhy (128). For non-coding DNA, we will estimate ζ (pronounced *zeta*), which compares the substitution rates of non-coding DNA that is upstream to transcription start-sites to substitution rates of synonymous codons (*dS*) or that of a nearby intron. Thus, we will test our predictions for Hypothesis A.

Transcriptomic Data

We will collect two blood samples from each *H. aureus*. One of these will be drawn into a DNA/RNA Shield™ vacuum tube (Zymo Research) and stored at -80°C, while the other sample will be processed using our metabolomic workflow (see below). We will collect samples from at least three separate animals for use as biological replicates in downstream analyses. After return to Kent State University, we will extract total RNA from each of these samples using an SV Total RNA Isolation System (Promega Corp.). We will then purify mRNA from these samples using a PolyATtract mRNA

Purification System (Promega Corp.) and convert these mRNA isolates to cDNA using a Tetro cDNA Synthesis Kit (Bioline). These processed samples will then be submitted to the NYU Genomics Core for RNA-seq library preparation and sequencing on their Illumina HiSeq 4000. The RNA-seq data for *L. catta* are publicly available through the Nonhuman Primate Reference Transcriptome Resource (98, 99).

After adapter- and quality-trimming the raw FASTQ data, we will use the RNA-seq data in two ways. **First,** they will be used to aid the annotation of our *H. aureus* and *L. catta* genome assemblies. Mentioned above, MAKER (119) can optionally map RNA-seq data to a given genome assembly in order to improve its gene prediction and annotation. Aside from only using MAKER, which uses BLASTn to accomplish this task, we will also use gapped-read alignment software (e.g., Bowtie 2: ref. 129) to map the reads to the annotated genome assemblies as a final quality-control check for MAKER's gene models. **Second,** we will then model the gene-expression profiles of whole blood from these samples using the RNA-seq count data using softwares designed for this purpose (e.g., DESeq2: ref. 130). These profiles will then be used to generate clustered heatmap-style comparisons (e.g., ref. 131) to find the major discriminants between the blood-specific expression profiles of *H. aureus* and *L. catta*. In conjunction with information about metabolic pathways, these results will allow us to test Prediction B1.

Metabolomic Data

For *L. catta,* we will purchase four fresh blood samples (i.e., from two males and two females) from the Duke Lemur Center. We will request that these samples be centrifuged prior to shipping in order to separate serum and hematocrit fractions, and we will store both of these, along with one half of the blood-serum samples from *H. aureus,* at -80°C until final analysis by gas chromatography-mass spectrometry (GC-MS) using an Agilent 5997A mass spectrometer equipped with an auto-injector and an Agilent 7890 gas chromatograph. The GC/MS analysis will be conducted at Northeast Ohio Medical University under the supervision of Dr. Takhar Kasumov and will focus on the detection of at least the following three molecules: cyanide, thiocyanate, and thiosulfate (i.e., the canonical substrate for rhodanese) in the blood serum of *H. aureus* and *L. catta*.

As is often the case with molecule detection by mass spectrometry (132, 133), we will not detect our three target molecules directly; rather, they will be transformed, or derivatized, in order to make them more stable or otherwise better suited for analysis by GC-MS. This follows the methods of others who have successfully used GC-MS to detect all three of our target molecules in biological samples (cyanide and thiocyanate: ref. 100; thiosulfate: ref. 134). Coincidentally, all three of these molecules can be derivatized by alkylation with the same molecule, pentafluorobenzyl bromide (PFB-Br) (135), and then using GC-MS to quantify levels of the transformed molecule. In the case of CN^- and SCN^-, the bromide ion in PFB-Br is displaced by either cyanide or thiocyanate, yielding PFB-CN or PFB-SCN, respectively, which can then be measured in the same GC-MS run (135). Thiosulfate ($S_2O_3^{2-}$), on the other hand, is processed by both alkylation and subsequent oxidation, leading to the formation of the analyte bis(pentafluorobenzyl)disulfide (134).

Prior to GC-MS analysis, we will add an internal standard of known concentration to samples in order to quantify levels of our target molecules. Following such sample preparation, we will then separate the samples by gas chromatography (which is a necessary step because of the diversity and complexity of molecules present in biological samples) and then immediately load the samples into the mass spectrometer. At this point, the sample will be ionized, accelerated, and finally detected. Because of the inclusion of an internal standard, the output of these runs will be able to be quantified and compared between the samples from *L. catta* and *H. aureus*, and these data allow us to test Prediction B2.

V. Pilot Data and Preliminary Research

Because of the primary role of sulfurtransferases in the detoxification of cyanide, we have conducted gene-targeted analyses into these genes in several species of bamboo lemur. In brief, this work indicates two preliminary findings: **First,** the cyanide adaptation of bamboo lemurs is not due to increased *dN/dS* ratios on the genes encoding rhodanese (*TST*) or mercaptopyruvate sulfurtransferase (*MPST*). **Second,** bamboo lemurs do not seem to be expressing rhodanese at a higher rate in the liver, which is one

of the two major organs where these genes are expressed in mammals (136–138). These results justify a more hypothesis-generating, "omic" approach, which we have begun by constructing a highly contiguous genome assembly for *Hapalemur griseus*. Additionally, we have submitted a sample of genomic DNA from a specimen of *H. aureus* for Illumina shotgun sequencing at an estimated read-depth of 20-30x.

To test for positive selection in the genes *TST* and *MPST*, we collected and aligned the CDS of these genes from 34 primate species (Fig. 3A). Thirteen of these derived from collaborator M. Elise Lauterbur's dataset, and the remainder were downloaded from NCBI Gene. We analyzed this robust dataset with PAML (127). Evaluating two alternative models for each gene (Fig. 3: see yellow and blue branches), we saw that the log-likelihoods of all alternative models were nearly identical those of the null models. To investigate this result, we constructed parameter-rich "free ratios models" (see ref. 139; Fig. 3). The reconstructed ancestral states revealed 68 nucleotide substitutions in the terminal branch for the *TST* ortholog of *Prolemur simus*, but the *dN/dS* ratio did not indicate positive selection (Fig. 3B: starred branch). We further examined this result by modeling 31 physicochemical properties of translated proteins with the program TreeSAAP (140). This analysis showed that the substitutions along the *Prolemur simus* branch for TST are likely conservative in nature: this branch showed significantly higher numbers of substitutions having the most minimal effect on a structural property known as beta-structure tendency (Fig. 4). Regarding *MPST*, the CDS for all bamboo-lemur orthologs showed high protein-level identity to that for *L. catta* (Fig. 2). Two branches appeared at first to indicate positive selection on *MPST* (Fig. 3B: double-starred branches). However, reconstructions of ancestral sequences showed that these high *dN/dS* ratios were artifacts caused by very low absolute numbers of substitutions (i.e., $N/S = 1/0$ in both cases). These preliminary findings suggest that the cyanide adaptation of bamboo lemurs is not due to positive selection on these sulfurtransferases.

We are currently investigating the relative expression levels of the sulfurtransferase enzymes rhodanese and MPST in the liver of captive *H. griseus* from the Duke Lemur Center (DLC). We extracted total RNA from the postmortem liver samples of captive *H. griseus, L. catta,* and *Propithecus coquereli* and converted this total RNA to cDNA. Then, we used a custom TaqMan® qPCR assay for *TST* to test the hypothesis that *H. griseus* expresses higher levels of rhodanese in its liver compared to a phylogenetic control, *L. catta*, and a dietary control species (i.e., folivorous lemuroid: ref. 141), *Propithecus coquereli*. We measured amplification data for the *TST* gene and a reference assay for eukaryotic18S rRNA using a QuantStudio 3 real-time PCR system. We compared the *TST* amplification data using the $\Delta\Delta C_T$ method (142), which is used to indicate fold changes in expression levels. **The preliminary results of this investigation indicate that *H. griseus* does not express higher levels than either of these controls**; quizzically, *L. catta* appears to express the highest levels of all three species (Fig. 5; Table 3) despite the fact that these *H. griseus* are fed a cyanogenic species of bamboo at the DLC (Erin E. Ehmke, pers. comm.). Considering these data, our provisional conclusion is that expression-level changes in the liver may not be responsible for the cyanide tolerance of bamboo lemurs. In practical terms, we take the negative results from this ongoing study and our positive-selection analyses as justification for the more hypothesis-generating genomic study outlined in this proposal.

We have obtained fresh blood draws from two animals currently housed at the DLC: a male *H. griseus* and a male *L. catta.* Using the 10X Genomics protocol for fresh frozen tissue, we extracted high molecular-weight genomic DNA from both of these samples (see ref. (143)) and submitted the *H. griseus* sample to the New York University Genomics Core Facility for high-throughput sequencing. The resulting *H. griseus* assembly, made with the Supernova assembler (109), showed very favorable summary statistics (Table 4). In particular, the N_{50} scores were several times higher than those for the recently published *Prolemur simus* genome assembly, which was sequenced to 152.7x coverage (contig N_{50} = 47.757 kb; scaffold N_{50} = 2.7 Mb). Furthermore, we recovered from this assembly 95.7% of mammalian BUSCOs (Table 4), a proportion similar to that of the *Prolemur simus* genome (97.7% BUSCOs recovered: ref. (115)), another primate genome that we have recently sequenced using the same method (Fig. 6), and other recent reference-quality genomes (e.g., refs. 144, 145).

VI. Intellectual Merit

This study will advance knowledge in two principal areas. First, it will be a significant investigation of the decades-old problem of how these primates have evolved to tolerate such high concentrations of cyanide. Several studies have addressed this topic (6–8, 146), but these studies have tended to focus on ancillary, but nonetheless important, details such as the cyanide content (6) or nutritional value (146) of the bamboo species consumed by these lemurs. Most relevant to the topic of the proposed project are two separate studies (8, 9) that have shown the urinary presence of cyanide or thiocyanate for several species of bamboo lemur. Because of such work, we can infer that bamboo lemurs are indeed absorbing into their bloodstreams prodigious amounts of cyanide, but no work has validated this inference or shown precisely how these lemurs have adapted to this extraordinarily toxic diet.

Second, this study will provide unique insights into cyanide toxicology, and these findings will very likely prove especially valuable because of the relatively close phylogenetic relationship of humans to lemurs (i.e., compared to most other model organisms that are the subjects of cyanide-based experiments: see refs. 82, 147). To date, some of the more effective cyanide antidotes utilize large amounts of biologically rare substances to remedy intoxication, such as hydroxocobalamin, which is a chemical precursor of the essential nutrient vitamin B_{12} (89, 148), or dicobalt EDTA, which relies on the rare metal cobalt to form stable cyanide-cobalt complexes (88). Other cyanide antidotes exhibit side effects that can have serious sequelae themselves. For example, antidotes whose active ingredient is amyl nitrite or sodium nitrite remove cyanide from the bloodstream by causing a conversion of normal hemoglobin to an oxidized form known as methemoglobin (148). This ameliorates cyanide poisoning but also lessens the oxygen-carrying capacity of blood because methemoglobin cannot bind and release oxygen (148). Because bamboo lemurs are coping with cyanide through some inborn and adaptive mechanism, and apparently not through supplementation of biologically rare substances, this study is poised to contribute insights for improving or developing therapies for cyanide intoxication.

VII. Broader Impacts

Aside from the principal objectives listed above, we are also committed to the following three goals related to public outreach and science education. **First,** we will invite and recruit undergraduates from minority groups underrepresented in STEM fields and train these students in many components of bioinformatic analysis. The PI has mentored three undergraduates from Kent State University's McNair Scholars Program, and the co-PI has trained and supervised these students during their time in the PI's laboratory. The goal of the McNair Scholars Program is to "prepare first-generation, low-income, undergraduate students, from groups underrepresented in graduate schools, for doctoral study" (149), and the PI and co-PI will both endeavor to recruit from this pool of hardworking undergraduates. The skills acquired by these students will include the following: DNA extraction, traditional PCR, quantitative PCR, sample preparation for high-throughput sequencing, and bioinformatic analysis.

The latter two goals will leverage existing connections of the co-PI in institutions that serve local communities. **Second,** we will present this project to the general lay public through the public libraries in the Northeast Ohio region. The co-PI has provided or assisted with programming for multiple libraries in his previous extracurricular involvement with local libraries (e.g., children's science programs). Public libraries also tend to serve minorities and underrepresented groups (150). **Third,** we will present this project and its findings to public schools in this same region to further stimulate interest in STEM fields. The co-PI is an alumnus of the AmeriCorps-supported organization Teach For America, which trains and supports teachers in low-income, high-need school districts, and he has acted as a judge for two science fairs for Akron Public Schools. Because of these connections, the co-PI would have relatively simple access to many science classrooms in Northeast Ohio.

VIII. Tables and Figures

Table 1: Diets of five bamboo lemur species. All data are were recorded by instantaneous sampling, also known as point sampling (151), and are percentages of the total number of feeding records.

Species	Study site	Duration of study	Bamboo and grass	Non-grassy foliage	Fruit	Other	Reference
H. alaotrensis	Lake Alaotra	15 months	95.4	2.0	0.0	0.3	(2)
H. griseus	Ranomafana National Park	2 years	88	4	5		(4)
H. griseus	Ranomafana National Park	12 months	90.7	5.8	1.2	2.3	(28)
H. meridionalis	Mandena Conservation Zone	62 hours[1]	76.0	21.3	1.9	0.8	(1)
H. aureus	Ranomafana National Park	2 years	88	3	4	5	(4)
P. simus	Ranomafana National Park	2 years	98	0	0.5	1.5	(4)

1: This number represents the number of complete hours of feeding data collected.

Table 2: Lethal doses of cyanide for various mammals.

Animal	Lethal dose[1] (mg CN^-/kg body weight)	Form of cyanide	Mode of exposure	Reference
Dog	1.3[2]	NaCN	Intravenous	(152)
	5.4	NaCN	Subcutaneous	(153)
Human	1.52[3]	various	Oral	(154)
Mouse	4.9 - 5.9	NaCN	Intraperitoneal	(152)
	< 6	KCN	Oral	(155)
Rabbit	2.3 - 2.7	various	Oral	(156)
	2.2[2]	NaCN	Subcutaneous	(152)
Rat	4.3	NaCN	Intraperitoneal	(152)
	3	NaCN	Oral	(157)
Hapalemur aureus	>>190[4]	Cyanogenic glycoside	Oral	(6)

1: Unless otherwise indicated, lethal dose is given as LD_{50}, or lethal dose of 50% of the sample population, in mg CN^-/kg body weight
2: Here, lethal dose is given as LD_{LO}, or the lowest observed lethal dose, also in mg CN^-/kg body weight
3: This estimate was calculated from a number of clinical case studies (cited in ref. (158)).
4: The figure given is not a lethal dose; rather it is the figure for the level of CN^- *ingested* by *H. aureus* from Ballhorn et al. (6). This was provided as about 4 x 1850 µmol/kg body weight. 1850 µmol CN^- converts to 48.13 mg.

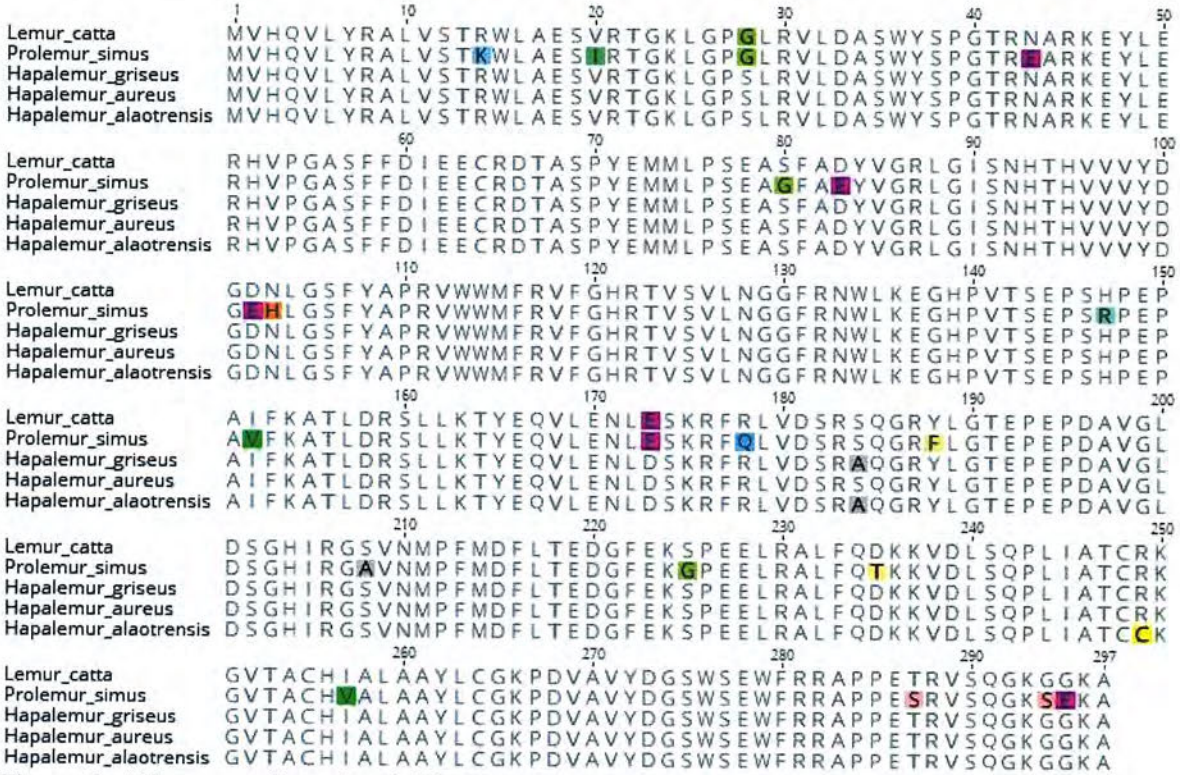


Figure 1: Alignment of translated *TST* CDS. The terminal stop codon has been removed from all sequences. All disagreements are highlighted.

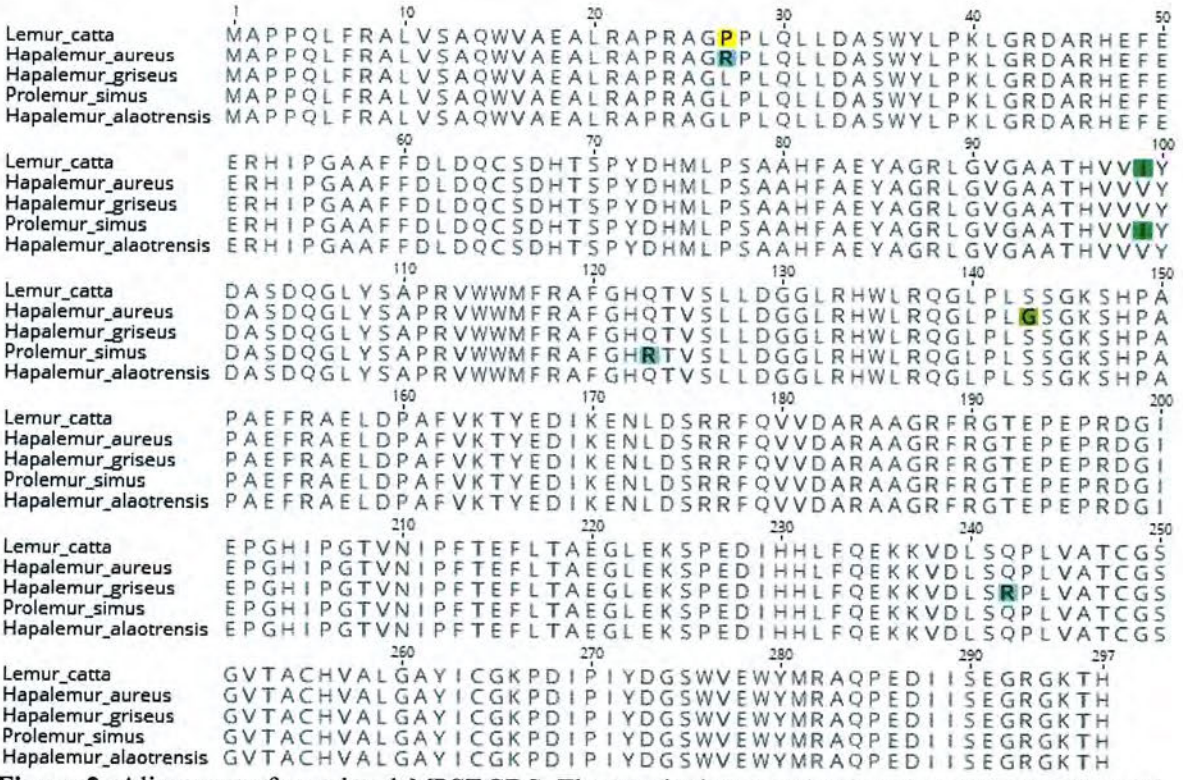


Figure 2: Alignment of translated *MPST* CDS. The terminal stop codon has been removed from all sequences. All disagreements are highlighted.

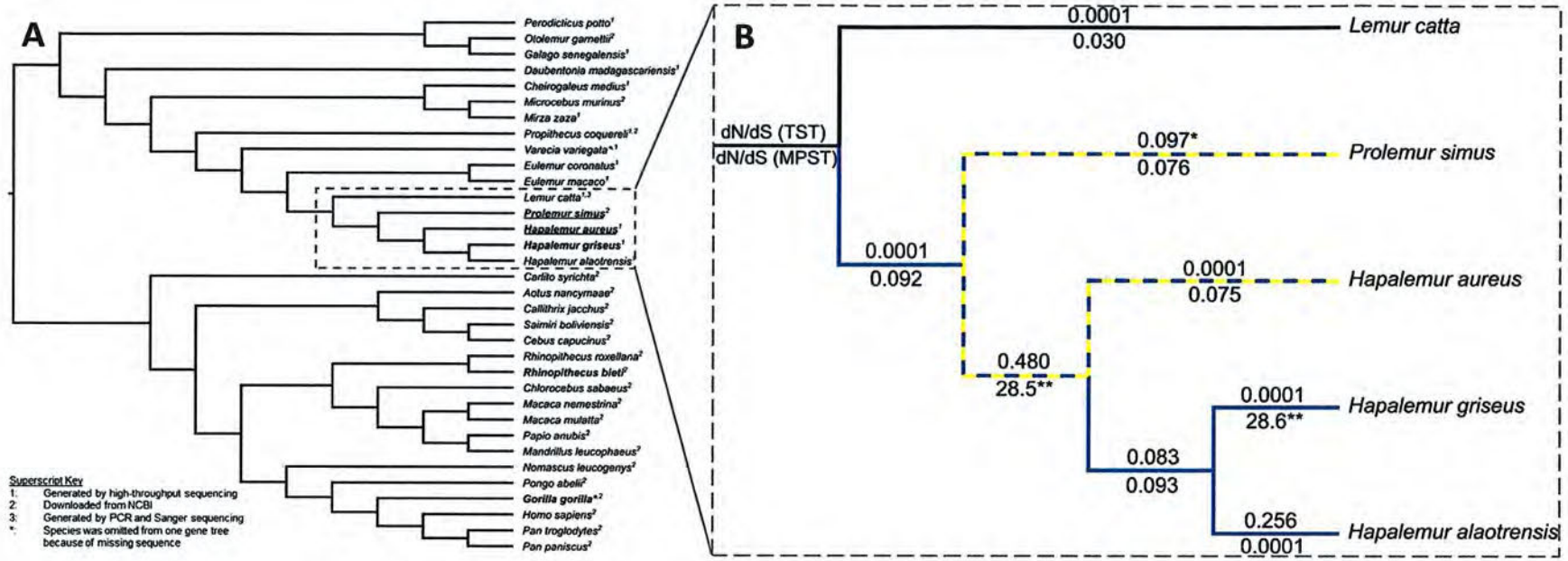


Figure 3: Branch-specific *dN/dS* estimates from PAML's free-ratio output. Branch with a single asterisk (*) displayed a high absolute number of substitutions (see Fig. 4). Branches with double asterisks (**) showed *N/S* ratios of 1/0. Bold text indicates that the species consumes high amounts of bamboo; underlined text indicates that the species is known to consume levels of cyanide that should be lethal.

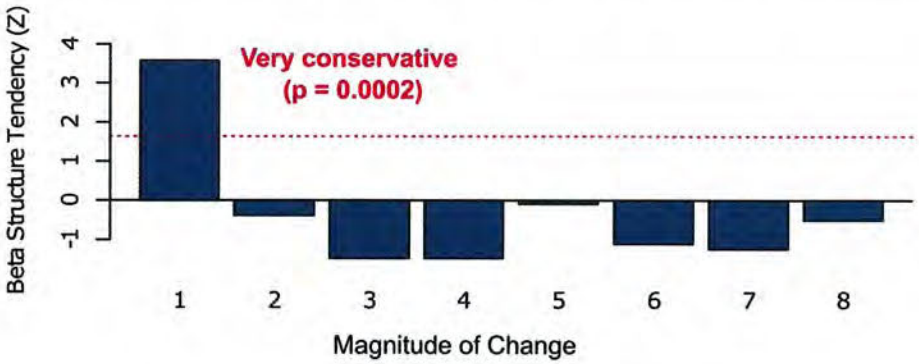


Figure 4: TreeSAAP results from the *TST* subtree depicted in the right pane of Figure 1. The red dashed line corresponds to $p = 0.05$ ($Z = 1.64$). Depiction of results follows McClellan et al. (ref. (159): their Fig. 1).

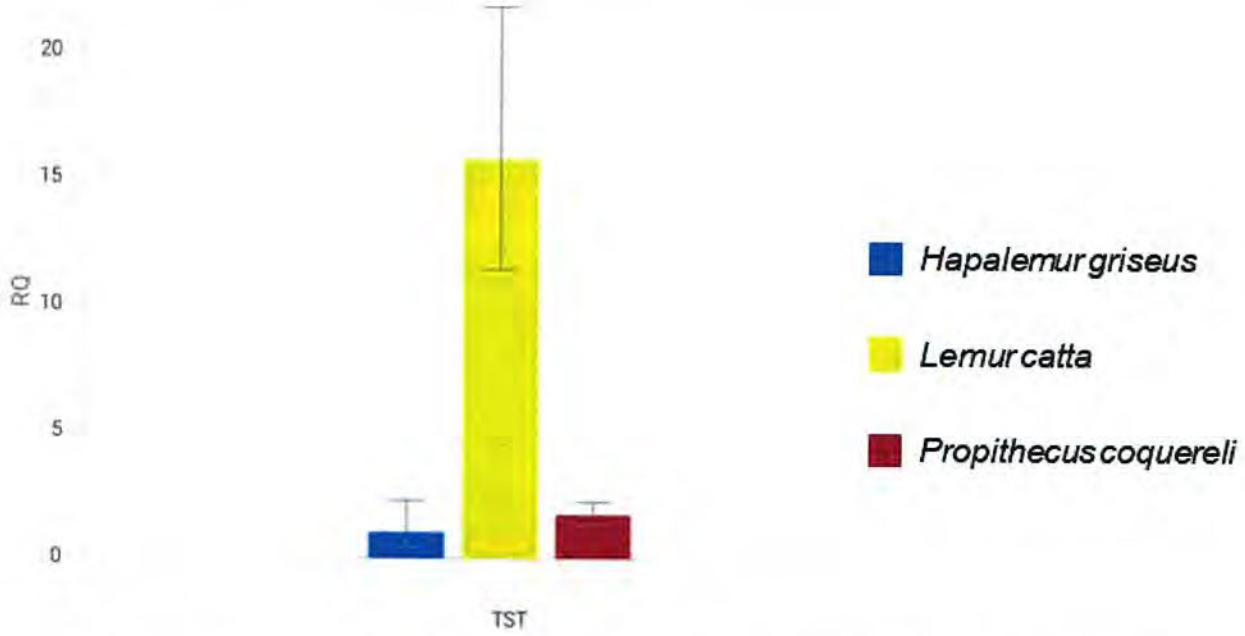


Figure 5: Relative expression levels of *TST* among livers of three lemuroid species. Relative quantification (RQ) = $2^{-\Delta\Delta Ct}$ (ref. (142)). For this comparison and in Table 3, *H. griseus* was set as the reference; therefore, its $\Delta\Delta C_T$ was set to 0.00, leading to a mean RQ of 1.00.

Table 3: Amplification data for target gene (*TST*) and reference gene (eukaryotic 18S rRNA). All species samples were sex-balanced, with the exception of *L. catta* (1 male, 2 females) due to sample availability.

Species (N)	**ΔC_T for 18S rRNA (SE)**	**ΔC_T for *TST* (SE)**	**$\Delta\Delta C_T$**	**RQ**	**RQ min.**	**RQ max.**
H. griseus (4)	14.135 (0.729)	22.149 (0.537)	0.000	1.000	0.441	2.268
L. catta (3)	16.218 (0.665)	18.179 (0.203)	-3.97	15.672	11.337	21.663
P. coquereli (4)	14.609 (0.533)	21.379 (0.171)	-0.77	1.705	1.314	2.214

Table 4: Select statistics from de novo assembly of *Hapalemur griseus* using the Supernova assembler (ref. (109))

Raw coverage	**Contig N_{50}**	**Scaffold N_{50}**	**Estimated genome size**	**BUSCOs recovered** (ref. (113))
69.49x	277.73 kb	20.47 Mb	2.61 Gb[1]	3927/4104 (95.7%)

1: This estimate falls within the range of other lemuroid genome assemblies currently available on NCBI Genome. *Microcebus murinus* (Mmur_3.0): 2.49 Gb; *Propithecus coquereli* (Pcoq_1.0): 2.80 Gb; *Prolemur simus* (KIAN8.4): 2.41 Gb.

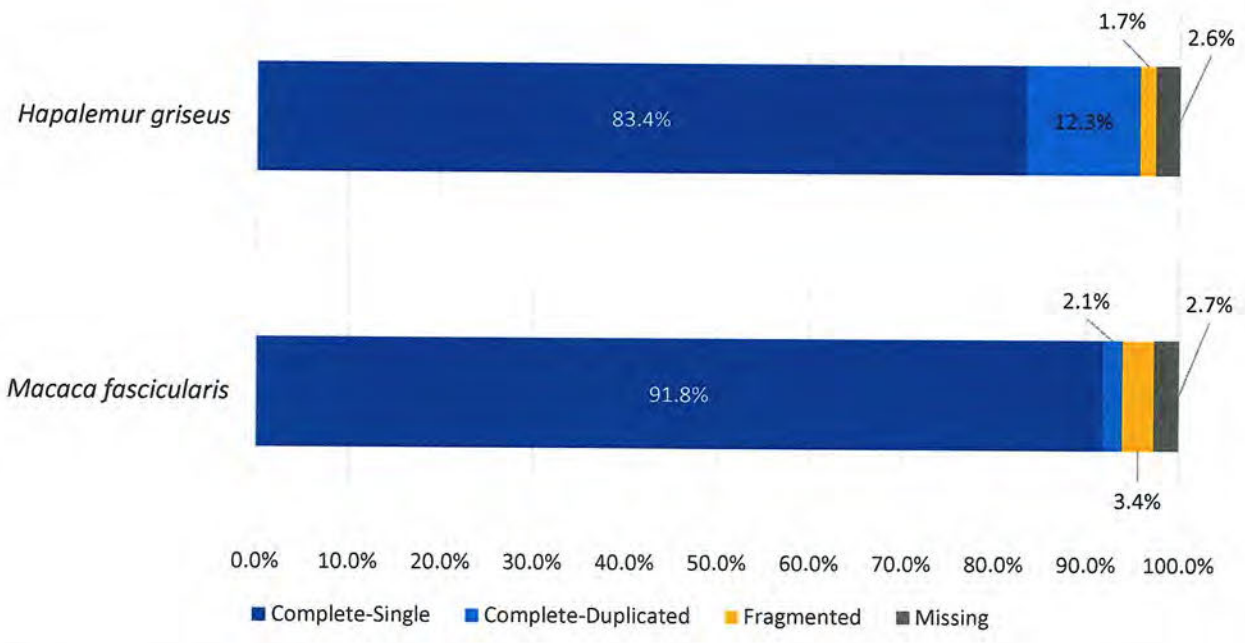


Figure 6: Number of Benchmark Universal Single-Copy Orthologs (113) recovered from two draft genome assemblies. Both of these assemblies were constructed using data obtained through the 10X Chromium method and assembled with the assembling algorithm Supernova (109).

IX. References

1. Eppley TM, Verjans E, Donati G (2011) Coping with low-quality diets: A first account of the feeding ecology of the southern gentle lemur, *Hapalemur meridionalis*, in the Mandena littoral forest, southeast Madagascar. *Primates* 52(1):7–13.

2. Mutschler T (1999) Folivory in a small-bodied lemur: The nutrition of the Alaotran gentle lemur (Hapalemur griseus alaotrensis). *New Directions in Lemur Studies*, eds Rakotosamimanana B, Rasamimanana H, Ganzhorn JU, Goodman SM (Kluwer Academic/Plenum Publishers, New York), pp 221–240.

3. Grassi C (2006) Variability in Habitat, Diet, and Social Structure of Hapalemur griseus in Ranomafana National Park, Madagascar. *Am J Phys Anthropol* 131:50–63.

4. Tan CL (1999) Group composition, home range size, and diet of three sympatric bamboo lemur species (genus *Hapalemur*) in Ranomafana National Park, Madagascar. *Int J Primatol* 20(4):547–566.

5. Glander KE, Wright PC, Seigler SS, Randrianasolo V, Randrianasolo B (1989) Consumption of cyanogenic bamboo by a newly discovered species of bamboo lemur. *Am J Primatol* 19:119–124.

6. Ballhorn DJ, Kautz S, Rakotoarivelo FP (2009) Quantitative Variability of Cyanogenesis in *Cathariostachys madagascariensis*—the Main Food Plant of Bamboo Lemurs in Southeastern Madagascar. *Am J Primatol* 71(4):305–315.

7. Ballhorn DJ, Rakotoarivelo FP, Kautz S (2016) Coevolution of Cyanogenic Bamboos and Bamboo Lemurs on Madagascar. *PLoS One* 11(8):e0158935.

8. Yamashita N, Tan CL, Vinyard CJ, Williams C (2010) Semi-Quantitative Tests of Cyanide in Foods and Excreta of Three Hapalemur Species in Madagascar. *Am J Primatol* 72:56–61.

9. Lauterbur ME, et al. (2017) Peeing Poison: The Biochemistry of Bamboo Lemur Cyanide Survival. *Integr Comp Biol* 57:E96.

10. Allen AR, Booker L, Rockwood GA (2015) Acute Cyanide Toxicity. *Toxicology of Cyanides and Cyanogens*, eds Hall AH, Isom GE, Rockwood GA (John Wiley & Sons, Ltd., Hoboken, NJ), pp 1–20.

11. Saito S, Tsukamoto S, Kanegae T, Oshida S, Nagoya T (2000) Influence of L-cysteine and hydoxocobalamin on cyanide distribution in rats. *Nihon Univ J Med* 42:215–228.

12. Ballantyne B (1975) Blood, Brain and Cerebrospinal Fluid Cyanide Concentrations in Experimental Acute Cyanide Poisoning. *J Forensic Sci Soc* 15(1):51–56.

13. Feldman JM, Feldman MD (1990) Sequelae of attempted suicide by cyanide ingestion: A case report. *Int J Psychiatry Med* 20(2):173–179.

14. Downey JD, Basi KA, DeFreytas MR, Rockwood GA (2015) Chronic cyanide exposure. *Toxicology of Cyanides and Cyanogens*, eds Hall AH, Isom GE, Rockwood GA (John Wiley & Sons, Ltd., Hoboken, NJ), pp 21–40.

15. Virion A, Deme D, Pommier J, Nunez J (1980) Opposite Effects of Thiocyanate on Tyrosine Iodination and Thyroid Hormone Synthesis. *Eur J Biochem* 112(1):1–7.

16. Bourdoux P, et al. (1978) Evidence that Cassava Ingestion Increases Thiocyanate Formation: A Possible Etiologic Factor in Endemic Goiter. *J Clin Endocrinol Metab* 46(4):613–621.

17. Manzano H, et al. (2007) Effects of long-term cyanide ingestion by pigs. *Vet Res Commun* 31:93–104.

18. Tylleskär T, et al. (1992) Cassava cyanogens and konzo, an upper motoneuron disease found in Africa. *Lancet* 339(8787):208–211.

19. Banea-Mayambu J-P, et al. (1997) Geographical and seasonal association between linamarin and cyanide exposure from cassava and the upper motor neurone disease konzo in former Zaire. *Trop Med Int Heal* 2(12):1143–1151.

20. Osuntokun BO (1980) Cassava diet, chronic cyanide intoxication and neuropathy in the Nigerian Africans. *World Rev Nutr Diet* 36:141–173.

21. Howlet WP, Brubaker GR, Mlingi M, Rosling H (1990) Konzo, an epidemic upper motor neuron disease studied in Tanzania. *Brain* 113:223–235.

22. Meier B, Albignac R, Peyriéras A, Rumpler Y, Wright PC (1987) A New Species of *Hapalemur* (Primates) from South East Madagascar. *Folia Primatol* 48:211–215.

23. Kappeler PM, Watts DP (2012) Long-term lemur research at Centre ValBio, Ranomafana National Park, Madagascar. *Long-Term Field Studies of Primates*, eds Kappeler PM, Watts DP (Springer-Verlag, Berlin, Heidelberg), pp 67–100.

24. Tan CL (2006) Behavior and ecology of gentle lemurs (Genus *Hapalemur*). *Lemurs: Ecology and Adaptation*, eds Gould L, Sauther ML (Springer Science+Business Media, LLC, New York), pp 369–382.

25. Eronen JT, et al. (2017) Feeding Ecology and Morphology Make a Bamboo Specialist Vulnerable to Climate Change. *Curr Biol* 27(21):3384–3389.

26. McKenney EA, Maslanka M, Rodrigo A, Yoder AD (2017) Bamboo Specialists from Two Mammalian Orders (Primates, Carnivora) Share a High Number of Low-Abundance Gut Microbes. *Microb Ecol*:1–13.

27. Eppley TM, et al. (2015) The use of an invasive species habitat by a small folivorous primate: Implications for lemur conservation in Madagascar. *PLoS One* 10(11):e0140981.

28. Overdorff DJ, Strait SG, Telo A (1997) Seasonal variation in activity and diet in a small-bodied folivorous primate, *Hapalemur griseus*, in southeastern Madagascar. *Am J Primatol* 43(3):211–223.

29. Mutschler T, Randrianarisoa AJ, Feistner ATC (2001) Population status of the Alaotran gentle lemur *Hapalemur griseus alaotrensis*. *Oryx* 35(2):152–157.

30. Di Fiore A, Link A, Campbell CJ (2011) The Atelines: Behavior and Socioecological Diversity in a New World Monkey Radiation. *Primates in Perspective*, eds Campbell CJ, Fuentes A, MacKinnon KC, Bearder SK, Stumpf RM (Oxford University Press, New York), pp 155–188. 2nd Ed.

31. Milton K (1980) *The foraging strategy of howler monkeys: A study in primate economics* (Columbia University Press, New York).

32. Fashing PJ (2011) African Colobine Monkeys: Their Behavior, Ecology, and Conservation. *Primates in Perspective*, eds Campbell CJ, Fuentes A, MacKinnon KC, Bearder SK, Stumpf RM (Oxford University Press, New York), pp 203–228. 2nd Ed.

33. Perrin MR (2013) The Gastrointestinal Anatomy of the Lesser Bamboo Lemur, *Hapalemur griseus*, with Comments on Digestive Function. *South African J Wildl Res* 43(1):79–83.

34. Campbell JL, Eisemann JH, Williams C V., Glenn KM (2000) Description of the gastrointestinal tract of five Lemur species: *Propithecus tattersalli, Propithecus verreauxi coquereli, Varecia variegata, Hapalemur griseus,* and *Lemur catta. Am J Primatol* 52(3):133–142.

35. Yamashita N, Tan CL, Vinyard CJ (2008) A comparison of gastrointestinal tracts of sympatric Hapalemur species. *Am J Phys Anthropol* Suppl.:226.

36. Davies D V., Hill WCO (1954) The abdominal portion of the alimentary system in *Hapalemur* and *Lepilemur. Proc R Soc Edinburgh, Sect B Biol* 65(2):182–204.

37. Van Soest PJ (1994) *Nutritional Ecology of the Ruminant* (Cornell University Press, Ithaca, NY). 2nd Ed.

38. Lambert JE (1998) Primate Digestion: Interactions Among Anatomy, Physiology, and Feeding Ecology. *Evol Anthropol* 7(1):8–20.

39. Chivers DJ, Hladik CM (1980) Morphology of the gastrointestinal tract in primates: Comparisons with other mammals in relation to diet. *J Morphol* 166(3):337–386.

40. Chivers DJ (1994) Functional anatomy of the gastrointestinal tract. *Colobine Monkeys: Their Ecology, Behaviour and Evolution*, eds Davies AG, Oates JF (Cambridge University Press, Cambridge), pp 205–227.

41. Milton K, McBee RH (1983) Rates of fermentative digestion in the howler monkey, *Alouatta palliata* (Primates: Ceboidea). *Comp Biochem Physiol Part A Physiol* 74(1):29–31.

42. Cabre-Vert N, Feistner ATC (1995) Comparative gut passage time in captive lemurs. *J Jersey Wildl Preserv Trust* 31:76–81.

43. Huang H, et al. (2016) Dietary resources shape the adaptive changes of cyanide detoxification function in giant panda (*Ailuropoda melanoleuca*). *Sci Rep* 6(September):1–11.

44. Jones DA (1998) Why are so many food plants cyanogenic? *Phytochemistry* 47(2):155–162.

45. Ballhorn DJ (2011) Cyanogenic Glycosides in Nuts and Seeds. *Nuts and Seeds in Health and Disease Prevention*, eds Preedy VR, Watson RR, Patel VB (Academic Press, Burlington, MA), pp 129–136.

46. Nelson L (2006) Acute Cyanide Toxicity: Mechanisms and Manifestations. *J Emerg Nurs* 32(4 (Suppl.)):8–11.

47. Borowitz JL, Isom GE, Baskin SI (2001) Acute and Chronic Cyanide Toxicity. *Chemical Warfare Agents: Toxicity at Low Levels*, eds Somani SM, Romano, Jr. JA (CRC Press, Boca Raton, FL), pp 301–319.

48. Brennan RJ, Waeckerle JF, Sharp TW, Lillibridge SR (1999) Chemical warfare agents: Emergency medical and emergency public health issues. *Ann Emerg Med* 34(2):191–204.

49. Salkowski AA, Penney DG (1994) Cyanide poisoning in animals and humans: A review. *Vet Hum Toxicol* 36(5):455–466.

50. Isom GE, Borowitz JL (2015) Cyanide-induced neural dysfunction and neurodegeneration. *Toxicology of Cyanides and Cyanogens*, eds Hall AH, Isom GE, Rockwood GA (John Wiley & Sons, Ltd., Hoboken, NJ), pp 209–223.

51. Soto-Blanco B, Maiorka PC, Górniak SL (2002) Effects of Long-Term Low-Dose Cyanide Administration to Rats. *Ecotoxicol Environ Saf* 53:37–41.

52. Sousa AB, Soto-Blanco B, Guerra JL, Kimura ET, Górniak SL (2002) Does prolonged oral exposure to cyanide promote hepatotoxicity and nephrotoxicity? *Toxicology* 174(2):87–95.

53. Solomonson LP (1981) Cyanide as a metabolic inhibitor. *Cyanide in Biology*, eds Vannesland B, Conn EE, Knowles CJ, Westley J, Wissing F (Academic Press, London), pp 11–28.

54. Erdoğan MF, Erdoğan G, Sav H, Gülllü S, Kamel N (2001) Endemic goiter, thiocyanate overload, and selenium status in school-age children. *Biol Trace Elem Res* 79(2):121–130.

55. Cipollone R, Ascenzi P, Tomao P, Imperi F, Visca P (2008) Enzymatic detoxification of cyanide: Clues from *Pseudomonas aeruginosa* rhodanese. *J Mol Microbiol Biotechnol* 15(2–3):199–211.

56. Cipollone R, Ascenzi P, Frangipani E, Visca P (2006) Cyanide detoxification by recombinant bacterial rhodanese. *Chemosphere* 63(6):942–949.

57. Isom GE, Borowitz JL (2015) Biochemical mechanisms of cyanide toxicity. *Toxicology of Cyanides and Cyanogens*, eds Hall AH, Isom GE, Rockwood GA (John Wiley & Sons, Ltd., Hoboken, NJ), pp 70–81.

58. Leavesley HB, Li L, Prabhakaran K, Borowitz JL, Isom GE (2008) Interaction of Cyanide and Nitric Oxide with Cytochrome c Oxidase: Implications for Acute Cyanide Toxicity. *Toxicol Sci* 10(1):101–111.

59. Baud FJ, et al. (2002) Value of lactic acidosis in the assessment of the severity of acute cyanide poisoning. *Crit Care Med* 30(9):2044–2050.

60. Zhang L, et al. (2010) Cyanide-Induced Apoptosis of Dopaminergic Cells Is Promoted by BNIP3 and Bax Modulation of Endoplasmic Reticulum-Mitochondrial Ca2+ Levels. *J Pharmacol Exp Ther* 332(1):97–105.

61. Way JL (1984) Cyanide intoxication and its mechanism of antagonism. *Annu Rev Pharmacol Toxicol* 24:451–481.

62. Kandel ER, Schwartz JH, Jessell TM (2000) *Principles of Neural Science* (McGraw-Hill, New York). 4th Ed.

63. Sun P, Rane SG, Gunasekar PG, Borowitz JL, Isom GE (1997) Modulation of the NMDA receptor by cyanide: enhancement of receptor-mediated responses. *J Pharmacol Exp Ther* 280(3):1341–1348.

64. Patel MN, Yim GKW, Isom GE (1992) Blockade of N-Methyl-D-Aspartate Receptors Prevents Cyanide-induced Neuronal Injury in Primary Hippocampal Cultures. *Toxicol Appl Pharmacol* 115:124–129.

65. Johnson JD, Conroy G, Isom GE (1987) Alteration of Cytosolic Calcium Levels in PC12 Cells by Potassium Cyanide. *Toxicol Appl Pharmacol* 88:217–224.

66. Kiang JG, Smallridge RC (1994) Sodium Cyanide Increases Cytosolic Free Calcium: Evidence for Activation of the Reversed Mode of the Na+/Ca2+ Exchanger and Ca2+ Mobilization from Inositol Trisphosphate-Insensitive Pools. *Toxicol Appl Pharmacol* 127:173–181.

67. Yang C-W, Borowitz JL, Gunasekar PG, Isom GE (1996) Cyanide-Stimulated Inositol 1,4,5-Trisphosphate Formation: An Intracellular Neurotoxic Signaling Cascade. *J Biochem Toxicol* 11(5):251–256.

68. Görlach A, Bertram K, Hudecova S, Krizanova O (2015) Calcium and ROS : A mutual interplay. *Redox Biol* 6:260–271.

69. Hüttemann M, et al. (2012) Regulation of mitochondrial respiration and apoptosis through cell signaling: Cytochrome c oxidase and cytochrome c in ischemia/reperfusion injury and inflammation. *BBA - Bioenerg* 1817(4):598–609.

70. Watabe M, Nakaki T (2007) ATP depletion does not account for apoptosis induced by inhibition of mitochondrial electron transport chain in human dopaminergic cells. *Neuropharmacology* 52:536–541.

71. Shou Y, Gunasekar PG, Borowitz JL, Isom GE (2000) Cyanide-Induced Apoptosis Involves Oxidative-Stress-Activated NF-κB in Cortical Neurons. *Toxicol Appl Pharmacol* 164:196–205.

72. Jones DC, et al. (2003) Cyanide Enhancement of Dopamine-Induced Apoptosis in Mesencephalic Cells Involves Mitochondrial Dysfunction and Oxidative Stress. *Neurotoxicology* 24:333–342.

73. Soto-Blanco B, Maiorka PC, Górniak SL (2002) Neuropathologic study of long term cyanide administration to goats. *Food Chem Toxicol* 40:1693–1698.

74. Blandini F, Porter RH, Greenamyre JT (1996) Glutamate and Parkinson's disease. *Mol Neurobiol* 12(1):73–94.

75. Ngudi DD, Kuo YH, Lambein F (2002) Food safety and amino acid balance in processed cassava "Cossettes." *J Agric Food Chem* 50(10):3042–3049.

76. Nassar NMA, Junior OP, Sousa M V., Ortiz R (2009) Improving Carotenoids and Amino-Acids in Cassava. *Recent Patents Food, Nutr Agric* 1:32–38.

77. Dohán O, et al. (2003) The Sodium/Iodide Symporter (NIS): Characterization, Regulation, and Medical Significance. *Endocr Rev* 24(1):48–77.

78. Chandra AK, Ray I (2001) Dietary Supplies of Iodine and Thiocyanate in the Etiology of Endemic Goiter in Tripura. *Indian J Pediatr* 68(5):399–404.

79. Fukuyama H, Nasu M, Murakami S, Sugawara M (1992) Examination of antithyroid effects of smoking products in cultured thyroid follicles: only thiocyanate is a potent antithyroid agent. *Acta Endocrinol* 127:520–525.

80. Rutkowski J V., Roebuck BD, Smith RP (1986) Liver damage does not increase the sensitivity of mice to cyanide given acutely. *Toxicology* 38(3):305–314.

81. Bhandari RK, et al. (2014) Cyanide toxicokinetics: The behavior of cyanide, thiocyanate and 2-amino-2-thiazoline-4-carboxylic acid in multiple animal models. *J Anal Toxicol* 38(4):218–225.

82. Sousa AB, Manzano H, Soto-Blanco B, Górniak SL (2003) Toxicokinetics of cyanide in rats, pigs and goats after oral dosing with potassium cyanide. *Arch Toxicol* 77:330–334.

83. Wood JL, Cooley SL (1956) Detoxication of cyanide by cystine. *J Biol Chem* 218(1):449–457.

84. Isom GE, Borowitz JL, Hall AH (2015) Cyanide metabolism and physiological disposition. *Toxicology of Cyanides and Cyanogens*, eds Hall AH, Isom GE, Rockwood GA (John Wiley & Sons, Ltd., Hoboken, NJ), pp 54–69.

85. Johnson JD, Isom GE (1985) The oxidative disposition of potassium cyanide in mice. *Toxicology* 37:215–224.

86. Fasco MJ, et al. (2007) Cyanide adducts with human plasma proteins: Albumin as a potential exposure surrogate. *Chem Res Toxicol* 20(4):677–684.

87. Logue BA, Hinkens DM, Baskin SI, Rockwood GA (2010) The Analysis of Cyanide and its Breakdown Products in Biological Samples. *Crit Rev Anal Chem* 40:122–147.

88. Hall AH (2015) Cyanide antidotes in clinical use: dicobalt EDTA (Kelocyanor®). *Toxicology of Cyanides and Cyanogens*, eds Hall AH, Isom GE, Rockwood GA (John Wiley & Sons, Ltd., Hoboken, NJ), pp 292–295.

89. Houeto P, Hoffman JR, Imbert M, Levillain P, Baud FJ (1995) Relation of blood cyanide to plasma cyanocobalamin concentration after a fixed dose ofhydroxocobalamin in cyanide poisoning. *Lancet* 346(8975):605–608.

90. Nagahara N, Ito T, Minami M (1999) Mercaptopyruvate sulfurtransferase as a defense against cyanide toxication: Molecular properties and mode of detoxification. *Histol Histopathol* 14(4):1277–1286.

91. Westley J, Adler H, Westley L, Nishida C (1983) The Sulfurtransferases. *Fundam Appl Toxicol* 3:377–382.

92. Westley J (1981) Cyanide and sulfane sulfur. *Cyanide in Biology*, eds Vennesland B, Conn EE, Knowles CJ, Westley J, Wissing F (Academic Press, New York), pp 61–76.

93. Herrera JP, Dávalos LM (2016) Phylogeny and Divergence Times of Lemurs Inferred with Recent and Ancient Fossils in the Tree. *Syst Biol* 65(5):772–791.

94. Simmen B, et al. (2006) Plant species fed on by *Lemur catta* in gallery forests of the southern domain of Madagascar. *Ringtailed Lemur Biology*, eds Jolly A, Sussman RW, Koyama N, Rasamimanana H (Springer Science+Business Media, LLC, New York), pp 55--68.

95. Goldman N, Yang Z (1994) A codon-based model of nucleotide substitution for protein-coding DNA sequences. *Mol Biol Evol* 11(5):725–736.

96. Wróbel M, Jurkowska H, Śliwa L, Srebro Z (2004) Sulfurtransferases and cyanide detoxification in mouse liver, kidney, and brain. *Toxicol Mech Methods* 14(6):331–337.

97. Wong WSW, Nielsen R (2004) Detecting Selection in Noncoding Regions of Nucleotide Sequences. *Genetics* 167:949–958.

98. Pipes L, et al. (2013) The non-human primate reference transcriptome resource (NHPRTR) for comparative functional genomics. *Nucleic Acids Res* 41(D1):906–914.

99. Peng X, et al. (2015) Tissue-specific transcriptome sequencing analysis expands the non-human primate reference transcriptome resource (NHPRTR). *Nucleic Acids Res* 43(D1):D737–D742.

100. Peralta J (2017) Identification and quantification of cyanide and its metabolites in lemur urine. Dissertation (City University of New York).

101. Struhsaker TT (2010) *The Red Colobus Monkeys* (Oxford University Press, New York).

102. Nowak K, Lee PC (2011) Consumption of cycads *Encephalartos hildebrandtii* by Zanzibar red colobus *Procolobus kirkii*. *J East African Nat Hist* 100(1–2):123–131.

103. Lambert RJ, Kindler BL, Schaeffer DJ (1988) The efficacy of superactivated charcoal in treating rats exposed to a lethal oral dose of potassium cyanide. *Ann Emerg Med* 17(6):595–598.

104. Cooney DO, Struhsaker TT (1997) Adsorptive capacity of charcoals eaten by Zanzibar red colobus monkeys: Implications for reducing dietary toxins. *Int J Primatol* 18(2):235–246.

105. Struhsaker TT, Cooney DO, Siex KS (1997) Charcoal consumption by Zanzibar red colobus monkeys: Its function and its ecological and demographic consequences. *Int J Primatol* 18(1):61–72.

106. Brightsmith DJ, Taylor J, Phillips TD (2008) The Roles of Soil Characteristics and Toxin Adsorption in Avian Geophagy. *Biotropica* 40(6):766–774.

107. Kikuchi Y, et al. (2012) Symbiont-mediated insecticide resistance. *Proc Natl Acad Sci U S A* 109(22):8618–8622.

108. McCormick SP (2013) Microbial Detoxification of Mycotoxins. *J Chem Ecol* 39(7):907–918.

109. Weisenfeld NI, Kumar V, Shah P, Church DM, Jaffe DB (2017) Direct determination of diploid genome sequences. *Genome Res* 27(5):757–767.

110. Warren RL (2016) RAILS and Cobbler: Scaffolding and automated finishing of draft genomes using long DNA sequences. *J Open Source Softw* 1(7):116.

111. Jones SJ, et al. (2017) The genome of the northern sea otter (*Enhydra lutris kenyoni*). *Genes (Basel)* 8(12). doi:10.3390/genes8120379.

112. Jones S, et al. (2017) The Genome of the Beluga Whale (*Delphinapterus leucas*). *Genes (Basel)* 8(12):378.

113. Simão FA, Waterhouse RM, Ioannidis P, Kriventseva E V., Zdobnov EM (2015) BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* 31(19):3210–3212.

114. Illumina (2018) Sequencing Coverage Calculator. Available at: https://support.illumina.com/downloads/sequencing_coverage_calculator.html [Accessed January 3, 2019].

115. Hawkins MTR, et al. (2018) Genome sequence and population declines in the critically endangered greater bamboo lemur (Prolemur simus) and implications for conservation. *BMC Genomics* 19(1):445.

116. Lischer HEL, Shimizu KK (2017) Reference-guided de novo assembly approach improves genome reconstruction for related species. *BMC Bioinformatics* 18(1):1–12.

117. Schneeberger K, et al. (2011) Reference-guided assembly of four diverse *Arabidopsis thaliana* genomes. *Proc Natl Acad Sci* 108(25):10249–10254.

118. Hammond SA, et al. (2017) The North American bullfrog draft genome provides insight into hormonal regulation of long noncoding RNA. *Nat Commun* 8(1):1–8.

119. Campbell MS, Holt C, Moore B, Yandell M (2014) Genome Annotation and Curation Using MAKER and MAKER-P. *Curr Protoc Bioinforma* 48:4.11.1-4.11.39.

120. Smit AFA, Hubley R, Green P RepeatMasker Open 4.0. Available at: http://www.repeatmasker.org.

121. Stanke M, Steinkamp R, Waack S, Morgenstern B (2004) AUGUSTUS: A web server for gene finding in eukaryotes. *Nucleic Acids Res* 32:309–312.

122. Korf I (2004) Gene finding in novel genomes. *BMC Bioinformatics* 5:1–9.

123. Lomsadze A, Ter-Hovhannisyan V, Chernoff YO, Borodovsky M (2005) Gene identification in novel eukaryotic genomes by self-training algorithm. *Nucleic Acids Res* 33(20):6494–6506.

124. The Gene Ontology Consortium (2000) Gene Ontology: Tool for the unification of biology. *Nat Genet* 25(1):25–29.

125. Carbon S, et al. (2017) Expansion of the gene ontology knowledgebase and resources: The gene ontology consortium. *Nucleic Acids Res* 45(D1):D331–D338.

126. Kanehisa M, Furumichc M, Tanabe M, Sato Y, Morishima K (2016) KEGG: New perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Res*:1–15.

127. Yang Z (2007) PAML 4: Phylogenetic Analysis by Maximum Likelihood. *Mol Biol Evol* 24(8):1586–1591.

128. Kosakovsky Pond SL, Muse S V. (2005) HyPhy: Hypothesis testing using phylogenies. *Statistical Methods in Molecular Evolution*, ed Nielsen R (Springer Science+Business Media, LLC, New York), pp 125–181.

129. Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. *Nat Methods* 9(4):357–359.

130. Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 15(12):1–21.

131. Khomtchouk BB, Hennessy JR, Wahlestedt C (2017) shinyheatmap: Ultra fast low memory heatmap web interface for big data genomics. *PLoS One* 12(5):e0176334.

132. Valdez CA, Leif RN, Hok S, Hart BR (2018) Analysis of chemical warfare agents by gas chromatography-mass spectrometry: Methods for their direct detection and derivatization approaches for the analysis of their degradation products. *Rev Anal Chem* 37(1):1–26.

133. Chauhan A (2014) GC-MS Technique and its Analytical Applications in Science and Technology. *J Anal Bioanal Tech* 5(6):1000222.

134. Kage S, Nagata T, Kudo K (1991) Determination of thiosulfate in body fluids by GC and GC/MS. *J Anal Toxicol* 15(3):148–150.

135. Bhandari RK, et al. (2012) Simultaneous determination of cyanide and thiocyanate in plasma by chemical ionization gas chromatography mass-spectrometry (CI-GC-MS). *Anal Bioanal Chem* 404(8):2287–2294.

136. Aminlari M (2006) Distribution of the cyanide metabolizing enzyme rhodanese in different tissues of domestic animals. *J Vet Pharmacol Ther* 26(Suppl. 1):128.

137. Aminlari L, Tavana M, Kazemipour N (2014) Rhodanese distribution in different tissues of rabbits. *Comp Clin Path* 23:1259–1262.

138. Aminlari M (2009) Distribution of the enzyme rhodanese in tissues of the cat (*Felis catus*). *J Feline Med Surg* 11(4):305–308.

139. Yang Z (1998) Likelihood ratio tests for detecting positive selection and application to primate lysozyme evolution. *Mol Biol Evol* 15(5):568–573.

140. Woolley S, Johnson J, Smith MJ, Crandall KA, McClellan DA (2003) TreeSAAP: Selection on amino acid properties using phylogenetic trees. *Bioinformatics* 19(5):671–672.

141. Irwin MT (2006) Ecologically enigmatic lemurs: The sifakas of the eastern forests (*Propithecus candidus, P. diadema, P. edwardsi, P. perrieri* and *P. tattersalli*). *Lemurs: Ecology and Adaptation*, eds Gould L, Sauther ML (Springer, New York), pp 305–326.

142. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. *Methods* 25(4):402–408.

143. 10X Genomics (2018) HMW DNA Extraction from Fresh Frozen Tissue. Available at: https://support.10xgenomics.com/genome-exome/sample-prep/doc/demonstrated-protocol-hmw-dna-extraction-from-fresh-frozen-tissue [Accessed January 11, 2019].

144. Johnson RN, et al. (2018) Adaptation and conservation insights from the koala genome. *Nat Genet*. doi:10.1038/s41588-018-0153-5.

145. Hulse-Kemp AM, et al. (2018) Reference quality assembly of the 3.5-Gb genome of *Capsicum annuum* from a single linked-read library. *Hortic Res* 5(1). doi:10.1038/s41438-017-0011-0.

146. Eppley TM, et al. (2017) High Energy or Protein Concentrations in Food as Possible Offsets for Cyanide Consumption by Specialized Bamboo Lemurs in Madagascar. *Int J Primatol* 38:881–899.

147. Aminlari M, Malekhusseini A (2007) Cyanide-metabolizing enzyme rhodanese in human tissues: comparison with domestic animals. *Comp Clin Path* 16:47–51.

148. Hall AH, Saiers J, Baud F (2009) Which cyanide antidote? *Crit Rev Toxicol* 39(7):541–552.

149. Kent State University (2018) McNair Scholars Program. Available at: https://www.kent.edu/mcnair [Accessed June 17, 2018].

150. Horrigan JB (2016) *Libraries 2016* Available at: http://www.pewinternet.org/2016/09/09/2016/Libraries-2016/.

151. Altmann J (1974) Observational Study of Behavior: Sampling Methods. *Behaviour* 49(3):227–266.

152. Sax NI (1984) *Dangerous Properties of Industrial Materials* (Van Nostrand Reinhold, New York). 6th Ed.

153. Chen KK, Rose CL (1952) Nitrite and thiosulfate therapy in cyanide poisoning. *J Am Med Assoc* 149:113–119.

154. Environmental Protection Agency (1987) Extremely hazardous substances list and threshold planning quantities: Emergency planning and release notification requirements (52 FR 13378). *Fed Regist* 52(77):13378–13410.

155. Ferguson HC (1962) Dilution of dose and acute oral toxicity. *Toxicol Appl Pharmacol* 4(6):759–762.

156. Ballantyne B (1983) The influence of exposure route and species on the acute lethal toxicity and tissue concentrations of cyanide. *Dev Toxicol Environ Sci* 11:583–586.

157. Ballantyne B (1988) Toxicology and Hazard Evaluation of Cyanide Fumigation Powders. *J Toxicol Clin Toxicol* 26(5–6):325–335.

158. Agency for Toxic Substances and Disease Registry (2006) *Toxicological Profile for Cyanide* (Atlanta, GA) doi:10.1201/9781420061888_ch68.

159. McClellan DA, et al. (2005) Physicochemical Evolution and Molecular Adaptation of the Cetacean and Artiodactyl Cytochrome *b* Proteins. *Mol Biol Evol* 22(3):437–455.

CURRICULUM VITAE
Morgan E. Chaney

Home Address:
[redacted]
Email: mchaney1@kent.edu

Work Address:
750 Hilltop Drive, Room 226
Kent, Ohio 44242
Phone: (330)672-4028

Formal Education

2021 *(projected)*	Doctor of Philosophy, Biomedical Science – Biological Anthropology. **Kent State University,** Kent, Ohio.
2015	Master of Arts, Anthropology. **Kent State University,** Kent, Ohio. Thesis: "Learning to live, or living to learn? Age-related differences in foraging ability, and the extended juvenility of *Cebus capucinus*."
2010	Bachelor of Arts *cum laude*, Anthropology. **Ohio University,** Athens, Ohio. Minors in Biological Sciences and German Language & Culture.
2006	Diploma with Honors. **Fairborn High School,** Fairborn, Ohio.

Other Training & Education

2011	Teach For America Summer Institute. **Delta State University**, Cleveland, Mississippi.
2010	Advanced Primate Behavior & Ecology. **La Suerte Biological Field Station.** Limón, Costa Rica.
2009 – 2010	Animal Caretaker. **American Primate Educational Sanctuary,** Blacklick, Ohio.
2008 – 2010	Undergraduate Research Immersion Program, Ohio Center for Ecology and Evolutionary Studies. **Ohio University,** Athens, Ohio.

Academic Positions

2019 – present	Graduate Teaching Assistant, Department of Anthropology. School of Biomedical Sciences, **Kent State University.**
Spring, 2019	Adjunct Instructor, Department of Biology. **Hiram College.**
2017 – 2019	Teaching Fellow, Graduate Student Orientation, Division of Graduate Studies. **Kent State University.**

2017 – 2019	Information Services Chair, Graduate Student Senate. **Kent State University.**
2015 – 2019	Graduate Teaching Assistant, Division of Preclinical Sciences, College of Podiatric Medicine, **Kent State University.**
2015 – 2016	Research Symposium Chair, Graduate Student Senate. **Kent State University.**
2015	Summer Research Fellowship, **Northeast Ohio Medical University.** PI: Rebecca Z. German, Ph.D.; directed by Christopher J. Vinyard, Ph.D.
2013 – 2015	Graduate Teaching Assistant, Department of Anthropology, **Kent State University.**
2011 – 2013	Seventh- and Eighth-Grade Science Teacher, **Palestine-Wheatley Middle School,** Wheatley, Arkansas.

Peer-reviewed Articles

1. **Chaney, M.E.,** Smith, C.S., Fredieu, J.R., Belovich, S.J., Siesel, K.J. (in press) "Novel branching pattern of the common fibular nerve: Emergence of the superficial fibular nerve within the anterior crural compartment." *J. Am. Podiatr. Med. Assoc.*
2. **Chaney, M.E.**, Piontkivska, H., Tosi, A.J. (2018) "Retained duplications and deletions of *CYP2C* genes among Primates." *Mol. Phylogenet. Evol.* 125: 204-212.
3. Meindl, R.S., **Chaney, M.E.**, Lovejoy, C.O. (2018) "Early hominids may have been weed species." *Proc. Nat. Acad. Sci. U.S.A.* 115(6): 1255-1249.
4. Janiak, M.C., **Chaney, M.E.**, Tosi, A.J. (2018) "Evolution of acidic mammalian chitinase genes (*CHIA*) is related to body mass and insectivory in primates." *Mol. Biol. Evol.* 35(3): 607-622.
5. Ruiz, C.A., **Chaney, M.E.**, Tosi, A.J. (2018) "Medical-grade buccal swabs versus drugstore cotton swabs: No difference in DNA yield." *MethodsX* 5: 39-42.
6. Key, A.J.M., Young, J.W., Fisch, M., **Chaney, M.E.**, Kramer, A., Eren, M.I. (2018) "Comparing the use of meat and clay during cutting and projectile research." *Eng. Fract. Mech.* 192: 163-175.
7. **Chaney, M.E.**, Brechtel, B.B., Dao, T.V., Belovich, S.J., Siesel, K.J., Fredieu, J.R. (2018) "The fibularis digiti quinti tendon: A cadaveric study with anthropological and clinical considerations." *The Foot* 34: 45-47.
8. **Chaney, M.E.** (2017) "Age-related differences in palm-fruit feeding and handling durations in *Cebus capucinus* on the Osa Peninsula, Costa Rica." *Neotropical Primates* 23(2): 41-45.

Manuscripts in Progress

1. Ruiz, C.A., **Chaney, M.E.**, Imamura, M., Imai, H., Tosi, A.J. "Predicted structural differences at four fertility-related Y-chromosome proteins in *Macaca mulatta*, *M. fascicularis*, and their Indochinese hybrids." Submitted to *Proteins*.
2. **Chaney, M.E.**, Romine, M.G., Piontkivska, H., Tosi, A.J. "Dynamic gene-family evolution among primate xenobiotic-metabolizing cytochrome P450 genes (*CYP1-3*)." In preparation.
3. **Chaney, M.E.**, Ruiz, C.A., Meindl, R.S., Lovejoy, C.O. "Comment on 'The African ape-like foot of *Ardipithecus ramidus* and its implications for the origins of bipedalism.'" In preparation for submission to *eLife*.

Published Abstracts

1. **Chaney, M.E.**, Romine, M.G., Piontkivska, H., Tosi, A.J. (March 30, 2019) "The dynamic evolution of the xenobiotic-metabolizing cytochrome P450 enzymes (*CYP1-3*) among primates." 88th Annual Meeting of the American Association of Physical Anthropologists. Poster presentation.
2. Ruiz, C.A., **Chaney, M.E.**, Tosi, A.J. (March 28, 2019) "Macaque Y-chromosome introgression: Proteomic analysis of four Y-genes between rhesus (*Macaca mulatta*) and cynomolgus (*M. fascicularis*) macaques." 88th Annual Meeting of the American Association of Physical Anthropologists. Podium presentation.
3. Brechtel, B.S., Dao, T.V., **Chaney, M.E.**, Belovich, S.J., Siesel, K.J., Fredieu, J.R. (April 22, 2018) "Findings of the anomalous fibularis digiti quinti muscle and implications of its associated variable presentations from cadaver dissections." Experimental Biology. Poster presentation.
4. Meindl, R.S., **Chaney, M.E.**, Lovejoy, C.O. (April 13, 2018) "Weed macaques provide insight into the demographic success of early hominids." 87th Annual Meeting of the American Association of Physical Anthropologists. Podium presentation.
5. **Chaney, M.E.**, Piontkivska, H., Tosi, A.J. (April 11, 2018) "Poison, primates, and cytochrome P450s: The evolution of xenobiotic-metabolizing enzymes among primates." 87th Annual Meeting of the American Association of Physical Anthropologists. Podium presentation.
6. **Chaney, M.E.**, Piontkivska, H., Tosi, A.J. (August 26, 2017) "Evolution of the *CYP2C* gene cluster among the Hominoidea." 40th Meeting of the American Society of Primatologists. Poster presentation.
7. Janiak, M.C., **Chaney, M.E.**, Tosi, A.J. (April 21, 2017) "A phylogeny of the *CHIA* gene in the context of insectivory." 86th Annual Meeting of the American Association of Physical Anthropologists. Podium presentation.
8. **Chaney, M.E.**, Ruiz, C.A., Hart, J.A., Hart, T.B., Detwiler, K.M., Tosi, A.J. (August 25, 2016) "Systematics of the Red Colobus Monkeys of the TL2 Region, Democratic Republic of Congo." Joint meeting of the International Primatological Society and the American Society of Primatologists. Podium presentation.
9. **Chaney, M.E.**, Norconk, M.A. (August 23, 2016) "Age-related differences in palm-fruit feeding and handling time in *Cebus capucinus*." Joint meeting of the International Primatological Society and the American Society of Primatologists. Poster presentation.

10. Ruiz, C.A., **Chaney, M.E.**, Tosi, A.J. (April 16, 2016) "Differences in DNA yield among buccal swab types: Medical-grade vs. standard cotton swabs." 85th Annual Meeting of the American Association of Physical Anthropologists. Poster presentation.
11. **Chaney, M.E.**, Ruiz, C.A., Hart, J.A., Detwiler, K.M., Tosi, A.J. (April 16, 2016) "Mitochondrial relationships of red colobus monkeys from the TL2 region (Tshuapa, Lomami, Lualaba River Basins), Democratic Republic of Congo, relative to other central African populations." 85th Annual Meeting of the American Association of Physical Anthropologists. Poster presentation.

Unpublished Abstracts

1. Meindl, R.S., **Chaney, M.E.**, Lovejoy, C.O. (March 30, 2019) "Sampling the biodemographies of macaque lineages provides new understanding of the success of early hominids." 88th Annual Meeting of the American Association of Physical Anthropologists. Poster presentation.
2. **Chaney, M.E.**, Lauterbur, M.E., Álvarez-Dávalos, L.M., Tosi, A.J. (October 27, 2018) "Cyanide-detoxifying enzymes show no evidence of positive selection in bamboo lemurs." 15th Annual Meeting of the Midwest Primate Interest Group. Poster presentation.
3. Ruiz, C.A., **Chaney, M.E.**, Tosi, A.J. (October 27, 2018) "Macaque Y-chromosome Introgression: A Comparison of Spermatogenesis Genes Between Rhesus (*Macaca mulatta*) and Cynomolgus (*Macaca fascicularis*) Macaques." 15th Annual Meeting of the Midwest Primate Interest Group. Podium presentation.
4. Meindl, R.S., **Chaney, M.E.**, Lovejoy, C.O. (October 27, 2018) "Sampling the biodemographies of macaque lineages provides understanding of the success of early hominids." 15th Annual Meeting of the Midwest Primate Interest Group. Podium presentation.
5. Brechtel, B.B., Dao, T.V., **Chaney, M.E.**, Belovich, S.J., Siesel, K.J., Fredieu, J.R. (November 4, 2017) "The fibularis digiti quinti tendon: A cadaveric study with anthropological and clinical considerations." Midwest Regional Meeting of the American Association of Anatomists. Poster presentation.
6. **Chaney, M.E.**, Piontkivska, H., Tosi, A.J. (October 21, 2017) "Duplication, deletion, and detoxification: The evolution of *CYP2C* genes among Primates." 14th Annual Meeting of the Midwest Primate Interest Group. Podium presentation.
7. **Chaney, M.E.**, Piontkivska, H., Tosi, A.J. (April 21, 2017) "Duplication and deletion of *CYP2C* genes among catarrhine primates." 32nd Annual Graduate Research Symposium, Kent State University. Poster presentation.
8. Ruiz, C.A., **Chaney, M.E.**, Tosi, A.J. (April 21, 2017) "The question of Y: A unique case of primate Y chromosome introgression in the context of mating competition." 32nd Annual Graduate Research Symposium, Kent State University. Poster presentation.
9. **Chaney, M.E.** (April 3, 2015) "Learning to live, or living to learn? Age-related differences in foraging behavior and extended juvenility in *Cebus capucinus*." 30th Annual Graduate Research Symposium, Kent State University. Podium presentation.

10. **Chaney, M.E.** (October 4, 2014) "Age-related differences in palm-fruit feeding and handling time in *Cebus capucinus.*" Midwest Primate Interest Group, Madison, Wisconsin. Poster presentation.
11. **Chaney, M.E.** (May, 2010) "Adaptations to folivory in primates." Annual Anthropology Student Symposium, Ohio University. Podium presentation.

Academic Service

- Teaching Assistant, Bioinformatics Workshop: "Introduction to Data Analysis in R for Primatologists." 2nd annual conference of the African Primatological Society. Entebbe, Uganda. September 2, 2019.
- Member, Student Technology Panel. Division of Information Technology, Kent State University. Spring, 2019.
- Member, Graduate Student Engagement Work Group. Division of Student Affairs, Kent State University. Spring, 2018 – present
- Dissector and videographer, Human Anatomy Video Dissection Project. Kent State University College of Podiatric Medicine. PI: Maria Sevilla. Spring, 2016 – Spring, 2017.
- Member, International Travel Award review committee. Kent State University Graduate Student Senate. Fall, 2016.
- Lead organizer, 31st annual Graduate Research Symposium. Kent State University Graduate Student Senate. Spring, 2016.
- Co-chair, *ad hoc* Student Committee. American Society of Primatologists. 2015 – 2017.
- Member, Research Award review committee. Kent State University Graduate Student Senate. Fall, 2015.
- Faculty advisor, Palestine-Wheatley Middle School Science Club. Fall, 2011 – Spring, 2013

University Teaching

1. *Quantitative Anthropology* (ANTH 38490), Kent State University. Taught as instructor of record. Fall, 2019.
2. Guest lecturer for *Human Gross Anatomy* (BSC 80111), Kent State University College of Podiatric Medicine. Fall, 2019.
3. *Evolution* (BIOL 33500), Hiram College. Taught as instructor of record. Spring, 2019.
4. *Human Gross Anatomy* (BSC 80111), Kent State University College of Podiatric Medicine, co-taught under supervision from Michael A. Landers, DDS and John R. Fredieu, PhD. Fall, 2015; Fall, 2016; Fall, 2017; Fall, 2018.
5. *Lower Extremity Anatomy* (BSC 80124), Kent State University College of Podiatric Medicine, co-taught under supervision from Kathy J. Siesel, DPM and Stephanie J. Belovich, PhD. Spring, 2016; Spring, 2017; Spring, 2018; Spring, 2019.
6. *Issues in Human Evolution* (ANTH 18631), Kent State University, laboratory course taught under supervision from Marilyn A. Norconk, PhD. Fall, 2013; Spring, 2014; Fall, 2014; Spring, 2015.

Awards & Funding

1. *(Pending)* Broad Agency Announcement – Life Sciences, U.S. Army Research Office. "Detecting genome-wide adaptations for cyanide tolerance." [redacted]
2. Biological Anthropology Program – Doctoral Dissertation Improvement Grant, National Science Foundation. "Investigating genomic and expression-level adaptations for cyanide detoxification in *Hapalemur aureus*." [redacted]
3. Research Award, Graduate Student Senate of Kent State University. "Optimizing a gas chromatography/mess spectrometry method for the detection of cyanide and its metabolites in primate blood serum." 3/18/2019 – 3/18/2020. [redacted]
4. University Fellowship, Kent State University Division of Graduate Studies. 5/13/2019 – 5/12/2020.
5. General Small Grant, American Society of Primatologists. "Interrogating expression levels of cyanide-detoxifying enzymes in the liver of *Hapalemur griseus*." 7/1/2018—11/6/2019 [redacted]
6. Research Award, Graduate Student Senate of Kent State University. "Constructing a whole-genome assembly for *Hapalemur griseus*." 3/21/2018—3/21/2019. [redacted]
7. Pollitzer Student Travel Award, American Association of Physical Anthropologists. Spring, 2018 [redacted]
8. Domestic Travel Award, Graduate Student Senate of Kent State University. Spring, 2018. [redacted]
9. Domestic Travel Award, Graduate Student Senate of Kent State University. Summer, 2017. [redacted]
10. Domestic Travel Award, Graduate Student Senate of Kent State University. Spring, 2016. [redacted]
11. Domestic Travel Award, Graduate Student Senate of Kent State University. Fall, 2014. [redacted]
12. Crowd-sourced Fundraising for M.A. Thesis, Experiment.com. June, 2014. [redacted]
13. Research Award, Graduate Student Senate of Kent State University. "Learning to live, or living to learn? Age-related differences in foraging ability, and the extended juvenility of *Cebus capucinus*." 1/1/2014—12/31/2014 [redacted]
14. Science Initiative for Middle Schools, St. Francis Community Foundation. Fall, 2012. [redacted]
15. Undergraduate Research Grant, Ohio Center for Ecology and Evolutionary Studies of Ohio University. Spring, 2009. [redacted]

Academic Societies

Midwest Primate Interest Group
American Association of Physical Anthropologists
American Society of Primatologists
Phi Beta Kappa, Lambda of Ohio

No. 09/19 MEDDD/SG/DIREDD/CFF238

REPORT OF OBSERVATION

Following the Research Authorization No. 214/19/MEDD/SG/AGEF/DGRNE issued Aug. 6 2019 in the name of Patricia Wright.
- The year two thousand nineteen the 28th of the month of August around four o'clock at the office of the Vondrozo station,

We the undersigned RAZAFINDRAVOTO Bernard, adjunct technical officer of Waters and Forests, officiated as the judiciary police head of the Environment and Sustainable Development station of the District of Vondrozo.

Assisted by:
- ANDRIANOELY Dina, researcher of Centre ValBio, Ranomafana

Have made the finding that the collection activities relative to the Research Authorization had the following results:

RESULT OF OBSERVATION

Date of observation: 1st, 2nd, 3rd, 4th, 5th, and 27th of August 2019
Research location: Near A.P. Mangaraba Mahasoa Fokontany Ivato CR Ivato District Vondrozo
Geographic location: S 22.42.222; E 47.28.376; Altitude: 249 m

Collection site	Sample	Types	Number of samples	Mode of preservation	Total	Estimated weight
Near A.P. Margarabaka Mahasoa	Blood (maximum 3ml per individual sample, 12 individual animals x 3 ml)	Whole blood	43	Small plastic tube, 2 ml, with preservation buffer to prevent coagulation, some frozen	67	215 g
		Buffy coat	12			
		Plasma	12			
		Protein	26	Small paper envelopes holding saver cards	26	100 g
	Tufts of fur		12	Small plastic tube, 2ml, frozen		40 g
	Ectoparasites		10	Plastic tube with 85% alcohol	10	800 g
	Feces		43	Plastic tube,	43	33 g 15 ml =

				14 ml (32) or 2 ml (11) with preservation solution		15g x 32g

We certify by the present observation that the collection activities were conducted in compliance with the technical and regulatory forest clauses.
The present Report of Observation is established for use.

Additions
-DGEF.DGRME and DREDD
For report

Signed in Vondrozo the 29th Aug. 2019
Agent of Observation
(Signed and sealed by RAZAFINDRAVOTO Bernard)

DEVELOPPEMENT DURABLE
ATSIMO ATSINANANA-ANOSY-ANDROY

CANTONNEMENT DE L'ENVIRONNEMENT
ET DE DEVELOPPEMENT DURABLE
VONDROZO

N°01 /19 MEDDD/SG/DIREDD/CFF238

PROCES-VERBAL DE CONSTATATION

Suivant l'Autorisation de recherche N° :214/19/MEDD.SG/AGEF/DGRNE en tale du 06 Aout 2019 au nm de Patricia Wrighi

-L'an deux mil dix-neuf le vingt-huit du mois d'Aout à partir de quatre heures au bureau de cantonnement de Vondrozo.

Nous soussigne RAZAFINDRAVOTO Bernard, Adjoint technique des Eau et Fords officié le Police judiciaire chef cantonnement de l'Environnement et de Développement Durable du District Vondrozo.

Assiste par :

-ANDRIANOELY Dina chercheur du centre VAUBIO Ranomafana

Avons procède à la constatation des activités de collecte relative à la dite autorisation de recherche dont les résultats sont les suivant :

RESULTAT DE CONSTATATION

Date de constatation : 01-02-03-04-05 et 27 Aout 2019

Localisation administrative : Hors d'A.P Mangaraba Mahasoa Fokontany IVATO CR IVATO District Vondrozo

Localisation géographique : S : 22 42 222 ; E : 047 28 376 ; Altitude : 249

Site du collection	Echantillon	Types	Nombre de Escarbillons	Mode du Préservation	Total	Poids estimatif
Hors A.P Margarabaka Mahasoa	Sarg (Au maximum 3ml par individu 12 indi x 3ml	Sang intact	43	Petit tube en plastique 2ml avec de la collection tampon de préservation d'acide pucleuque pais congelés	67	215 g
		Couche leuco plaquettaire	12			
		Plasma	12			
		Protéine	26	Petit enveloppes en papier portier saver card	26	100 g
	Touffe en poids		12	Petit tube enplaision de 2 ml poids congelés		40 g
	Ectoparasites		10	Tube en plastics de sons avec de l'acol 85°	10	800 g
	Feeces		43	Tube en plastique de 14 ml (32) ou de 2ml (11) avec de solution de presse notation	43	33 g 15ml= 15g x 32g

Nous certifiant par le présents procès-verbal que les activités de collecte ont été farte dans le respect des clauses techniques et réglementaire en matière forestiers

Le présent Procès-verbal de constatation est établi pour être servir aux fins utiliser.

Le Chef Cantonnement de l'Environnement de l'Ecologie et des Forêts VONDROZO

Fait à Vondrozo, le 29 Aout 2019

l'Agent de Constatation

RAZAFINDRAVOTO Bernard
Adjoint Technique des Eaux et Forêts

AMPLIATION

-DGEF.DGRME et DREDD

Pours compte-rendu

PROPOSAL FOR TRANSLOCATION AND REINTRODUCTION OF *PROLEMUR SIMUS* TO THE FOREST OF TALATAKELY IN RANOMAFANA NATIONAL PARK

Presented by the Department of Biological Anthropology and Sustainable Development and ICTE/CVB and MNP
Represented by Dr. Patricia Wright
patchapplewright@gmail.com

Background

Prolemur simus, formerly known as *Hapalemur simus* Groves, 2001), is by far the most threatened species of lemurs in Madagascar (Ganzhorn et al., 1996/1997; Mittermeier et al., 2010 Wright, et al., 2008). Data from known subfossils showed that this species was widely distributed in Madagascar, Anjohibe and Ankarana massifs in the north, caves in the Bemaraha Tsingy in the west and even in the highlands in Ampasampazimba, 25 km to West of Antananarivo (Godfrey et al., 2004; Godfrey and Vuillaume-Randriamanantena, 1986; Simons, 1997). The present *P. simus* populations are found in the humid forests of the South East. Prior to the 1970s, *P. simus* was known at only two sites, the Kianjavato coffee plantation and the Vondrozo forest (Meier et al., 1987; Meier and Rumpler, 1987; Petter et al., 1977; Wright et al. 1987). After some extensive research and destruction of the forest, this species was suspected to be extinct (Godfrey and Vuillaume-Randriamanantena, 1986), but the two research teams that arrived in Madagascar in June 1986 found individuals from this species. A group of 12 *P. simus* individuals were observed in the Kianjavato coffee plantation (Wright et al., 1987), and probably the same group (N = 6 individuals) was observed at the same location several months later (Meier et al., 1987; Meier and Rumpler, 1987). In addition, a second group of 11 individuals was observed in the Ranomafana classified forest (Meier et al., 1987; Wright, 1988; Wright et al., 1987). One of the reasons for protecting this forest as a National Park was the presence of two rare species, *Hapalemur aureus,* a new species, and *P. simus* (Wright, 1992; Wright and Andriamihaja, 2003).

The South East Census Report has confirmed that *P. simus* is severely threatened (Wright et al., 2008) and this report has inspired censuses in other areas. New groups have been discovered at sites such as Ranomainty and Sakalava of the Ankeniheny-Zahamena Corridor and the Vohiposa region near Brickaville (Randriarimanana, et al., 2012, Feaniaina, et al., 2012, Anjara et al., 2012) , the North East part of the Kianjavato Forest, the fragment of Vohitrarivo (Tsaratanana), and in the South part of Ivato, Karianga. (However, the only groups in a protected area are the groups in Ranomafana National Park).

Hapalemur aureus and *Prolemur simus* are the symbols of Ranomafana National Park. Long-term studies of these animals have shown that the number of individuals in the P*rolemur simus* group at the Talatakely site in Ranomafana National Park has declined. Indeed, the Talatakely site presents some groups of *Prolemur simus* but this group is the only one that is used by tourists and researchers. This group currently has only two individuals (father and daughter), with over 10 individuals at the beginning of the observations ten years ago (Wright et al., 2008). On the other hand, follow-ups and reconnaissance around the Park have identified groups of *Prolemur simus* in the Ivato forest fragment. We therefore thought that translocating individuals from this group to the Talatakely group would be a way to increase the number of *P. simus* groups so that they can reproduce without inbreeding. This reintroduction project was initiated in collaboration with Regional Ministry of Environment and Sustainable Development officials, Madagascar National Parks officials and ValBio Center agents and scientists in September 2012. Fifteen individuals (three male and twelve females) from the group outside the Park in the unprotected Karianga fragment will be captured and introduced into the

Talatakely group. As theis group is at a considerable distance from the Talatakely group, we expect success in accepting new individuals as potential mates and then reproduction.

The decline in the number of individuals in the protected area was discussed at the IUCN meeting in Antananarivo in July 2012, and everyone agreed that this would be a good strategy. The success of the Aspinall Foundation Project in the Ankeniheny-Zahamena Corridor (CAZ) and Brickaville's project to protect new northern P. simus groups through communities has been discussed and accepted as a good strategy; however, we need more than one approach to safeguarding these severely threatened animals (Randriarimanana, et al., 2012, Felaniaina, et al., 2012, Anjara et al., 2012). We have much more knowledge about *Prolemur simus*: this critically endangered species does not reproduce well in captivity (Rouillet, 2012) and the translocation of young individuals from endangered populations could be a better approach for the conservation of this species.

Introduction

The conservation problem raised in this proposal concerns a critically endangered species, *Prolemur simus*, which has long been found in Talatakely in Ranomafana National Park. *Prolemur simus* is a flagship species in the Park and attracts many tourists and researchers. The existence of this species was among the selection criteria for the World Heritage Site Park in 2007. In addition, a decrease in the number of *P. simus* groups and individuals was observed in the Talatakely Forest in 2006 (Wright et al., 2008). Wright et al. (2008) drew to the attention of researchers and non-governmental organizations working in the field of environment and conservation that there were only 100 known individuals of *P. simus* in the world. Based on this information, research on all remaining *P. simus* populations has begun in regions likely to find them. *P. simus* populations have been found in Ivato near Farafangana, as well as in Torotorofotsy and around Ambatovy near Andasibe. In 2008, approximately 1 km outside the Ranomafana National Park boundary, *P. simus* groups were located in Vohitrarivo (groups of 45 and 48 individuals), and in Sahofika (around 35 individuals). In 2008 the ValBio Center research team were able to observe three new *P. simus* groups in the fragmented forest of Ivato, near Karianga. Four individuals were briefly sighted in Antanifotsy in 2010 and this group was tracked during the HELP Simus Conservation Project.

In 2010, the ValBio Biodiversity Team, monitoring the Ivato Forest, discovered three different groups (31, 12, 26 individuals respectively). We worked closely with the village of Ivato in creating tree nurseries and hired local residents in 2009-2010 to follow the animals. The study continued until 2015. Thanks to the CVB's collaboration with the village of Ivato, *P. simus* were not injured and the population increased, becoming 46, 16 and 50. The people of Ivato complained about *P. simus* eating their crops (sugar cane, coffee, rice and cassava) and asked us to remove some *P. simus* individuals.

Description of *Prolemur simus*

The genus *Hapalemur*, of which *Prolemur simus* was formerly considered a member, are known as bamboo lemurs because of their primarily bamboo diet. There are seven species and sub-species with different adaptive characteristics, including *H. meridionalis*, *H. aureus*, *H. griseus occidentalis*, *H. griseus ranomafanensis, H. griseus griseus*, and *H. simus* (*Prolemur simus*) (Mittermeier et al, 2011). Like other lemurs, these species are threatened with extinction due to the dramatic habitat degradation, due to excessive human activities. This includes direct hunting for sale or food; logging large trees to use for building and woodworking, and slash-and-burn agriculture (tavy). As a result, forests become open, fragmented, shrink, lose many plants species used by animals for food, such as bamboos, and the forest is replaced by "savoka" (cropland).

For *Prolemur simus* the situation is alarming as it is only present in 1-4% of its historical range and studies show that it is a food and habitat specialist. It eats principally one species of bamboo, *Cathariostachys madagascariensis*, which is also sensitive to edge effects and prefers forest interiors (Petter et al, 1977). Hence the population of *P. simus* has become very small, about 12 groups, made up of about 100 individuals, are known in their current distribution. *Prolemur simus* is gravely threatened (Wright et al, 2008). Thus captive management and *in situ* population management are recommended to save the rest of the population (CAMP, 2001; Mittermeier et al, 2011; IUCN, 2012).

For *Prolemur simus ex situ* conservation, only 39 individuals are being held captive in 22 institutions in Madagascar and in Europe, namely Ivoloina Park, Paris Park, Column Park, etc. As for *in situ* conservation, efforts have been made to systematically inventory the sites, including genetic study and other research in both its historic habitat and other sites. According to previous studies, the historic distribution of *Prolemur simus* was the northern, western, northwestern, central and eastern portion of Madagascar (Mittermeier et al. 2008). Currently this species is found only in the humid forest of South East and Central East Madagascar. In the south, it is present in Ranomafana National Park, Andringitra National Park, and five other unprotected forests including Kianjavato, Karianga, Morafeno, Evendra, Mahasoa (Wright et al, 2008). In the center it is found in the Torotorofotsy forest near the Ambatovy mining site (Dodge 2005, King et al. 2013).

Now, in the small *Prolemur simus* population in Ranomafana National Park, about 20 individuals in 3 groups disperse to and from the Talatakely group, which is the only group accustomed to tourists and researchers. In this group there is only one adult male and his daughter, a young female. The goal of this reintroduction project is the recovery of this population in Ranomafana National Park, which is the only population within a protected area, by introducing individuals from another region to form stable, reproductive groups that ensure population viability within the Park. The main objectives are to improve genetic diversity to prevent inbreeding and population extinction and to maintain ecosystem balance. Indeed, the project would consist of capturing *Prolemur simus* from the Ivato forest, and reintroducing it to the Talatakely site within Ranomafana National Park.

Hypotheses for the disappearance of *Prolemur simus* at Talatakely

The reason for the decrease in the number of groups and individuals in the Talatakely forest have not yet been confirmed. We consider four possible hypotheses, including habitat and nutrition degradation, predation, inbreeding, and stress from ecotourism.

Habitat and nutrition degradation

In Ranomafana National Park, 95% of the *Prolemur simus* diet consists of the bamboo *Cephalostachyum madagascarensis* (giant bamboo). *Prolemur* eat mature leaves, young leaves, stems, pith, and young shoots of *C. madagascariensis* (Tan, 1999, 2000). Young shoots contain a large amount of protein and cyanide (Glander et al., 1992, Balhorn et al., 2010). The counting of bamboo stems in botanical plots carried out in 1997 at Talatakely showed a relative *C. madagascariensis* abundance that varies from 0-36.6% (Tan, 1999). In 2012 we re-evaluated the number of *C. madagascariensis* stems of at Talatakely and found that their relative abundance ranged from 5-40%. These data show that bamboo abundance at Talatakely has not decreased over the past 14 years and that could mean that b**amboo abundance is not the reason for the decrease in numbers of *P. simus* groups and individuals in the park.**

One of the regions (10 ha) occupied by giant bamboo in the Talatakely forest where Prolemur simus frequent.

Another region (9.8 ha) occupied by bamboo in Talatakely forest, also frequented by P. simus

Predation

The main predator of lemurs is *Cryptoprocta ferox*, the Fosa. During 26 years of studying lemurs in Ranomafana, the Fosa has been recognized as a predator of *Eulemur rubriventer, Propithecus diadema (edwardsi),* and *Varecia variegata.* We do not have evidence of predation of *Prolemur simus* by Fosa. Analysis of camera trap data estimates 25 *C. ferox* in Ranomafana National Park (41,600 ha) (Gerber et al., 2012). In other sites, Fosa eat Sifakas from small forest fragments (Irwin et al., 2010); during the 26 years of continuous study of *Propithecus edwardsi* in Ranomafana National Park, there have been 14 known cases of predation by Fosa (Wright 1998, Irwin et al., 2010). It has been hypothesized that the high cyanide content (a toxin that kills humans) of bamboo eaten by *P. simus* could deter predators. Since the group of *P. simus* at Talatakely has been followe for at least five days a week for the past 17 years, if *C. ferox* had killed *P. simus*, researchers would have found the remains, as is the case in other lemur species studied in this location. Thus, **predation is excluded as the reason for the decrease in the number of groups and individuals of *P. simus* at Talatakely.**

Inbreeding

In the past, this group of *P. simus* included a mother and her daughter and granddaughter as breeding females. Male offspring left the group at the age of 3-5 to have breeding opportunities in the other

groups. When a breeding male that joined the group in 1999 disappeared in 2005, these females and their offspring emigrated to find another male. Currently, the only individuals in this group of *P. simus* are the father and daughter, who have not shown any inclination to breed with each other. In this case, even if there is reproduction, the risk of detrimental effects of inbreeding would be very high. Therefore, the probability of emigration of these two remaining individuals is very high as well. **The lack of genetic diversity could be, then, one of the reasons for the disappearance of *P. simus* individuals, hence the decrease in the number of groups and individuals at Talatakely.** It is thus appropriate to make the decision to translocate a few individuals from other sites to add genetic diversity to the *P. simus* group in the Talatakely Protected Area.

Effects of tourism (stress and frequent human presence)

We conducted a short study in 2011 asking tourists the main reasons for their visits to Ranomafana National Park. This study shows that lemurs, in particular, *Hapalemur aureus*, *Propithecus edwardsi* and *Prolemur simus*, are the main reason for their visit to the Park. Many tourists want to see *Prolemur simus*, which is a critically endangered species. 84% of tourists (n = 245) saw *Prolemur simus* in 2011 and expressed satisfaction with the visit to Ranomafana. However, this frequent and abundant human presence could cause stress to the animals as well as trample future bamboo shoots. Could it be, then, one of the reasons for the disappearance of *P. simus*? If so, then a comparison of *P. simus* groups in areas more frequented by humans should be performed compared to those less frequented by people using behavioral and hormonal indicators of stress. *Evidence of the effect of tourism on the disappearance of Talatakely's Prolemur simus is complex and deserves more in-depth studies in the medium or long term.*

Based on the four hypotheses proposed above, the lack of genetic diversity and the risk of inbreeding among the individuals in the groups could be causes of the decrease in the number of *P. simus* groups and individuals at Talatakely. Therefore, we strongly suggest to the Ranomafana National Park Manager that a few *P. simus* individuals be translocated from the nearest site with a similar habitat in order to reintroduce them into the Talatakely Forest to save the current population and maintain genetic diversity.

Description of previous translocation and lessons learned

In 2014, the CVB team transferred three *Prolemur simus* to Ranomafana National Park. However, this translocation attempt was not successful because none of the three individuals remained in the area. They could not be followed because their radio collars fell off.

The potential reasons for this failure as well as the recommendations for success in this new attempt will be listed below.

The criterion for success is the creation of a new group of *P. simus* within the range of the current group in Ranomafana National Park, and, eventually, the dispersal of sime individuals to the existing group (father and daughter).

Error: We captured three individuals for translocation
Recommendations: Experts advise that at least 6 to 16 individuals be transferred to succeed.
2019 translocation project: We are going to transfer at least 6 individuals, but we hope that 16 individuals from the Ivato site will be transferred to the Ranomafana National Park.

Error: We captured three individuals belonging to three different groups of *P. simus*
Recommendations: All captured individuals must belong to the same group.
Translocation 2019 project: We will transfer together all individuals from the same group

Error: We did not observe the individuals before translocation.
Recommendations: Individuals must be followed to observe the relationships and health for at least 10 days to two weeks.
Project translocation 2019: We will follow individuals for ten days to two weeks before capture to choose the best individuals to transfer.

Error: We left the 3 individuals in the habituation cage overnight in Ranomafana National Park, and then released them the next day.
Recommendations: Individuals should be allowed to get used to the inside of the cage for about 2 to 4 weeks to allow them to adapt to the environment.
Project Translocation 2019: We will keep individuals transferred to the cage for at least two weeks, feeding them with bamboo and other foods three times a day.

Error: The size of the radio collars used was too big and they fell off
Recommendations: Collar size should be appropriate for each individual
Translocation Project 2019: We will place appropriate collars on each individual to follow them after release.

We will ensure that each animal feeds properly. We will also monitor the parasite load and animal health during the two weeks of habituation in the Park. We will separate animals that get sick or do not eat well in a smaller cage and monitor them more closely.

Release: Once the translocated individuals are released, they will be followed for ten consecutive days by rotating teams of CVB observers. Sixteen technicians will be employed to track each individual transferred from dawn to dusk, taking data on behavior, diet, interactions with other individuals.

Follow-up: after the ten-day period, these animals will be observed five days a week. A weekly report will be given to MNP and CVB. Any reproduction and/or mating behavior observed will be noted.

Importance of Translocation

Although we have been monitoring *P. simus* populations in Ivato intermittently since 2008, local communities have recently asked us to remove some animals in order to reduce the number of animals in the area because the animals are destroying their crops. So far, we have maintained good relations with the villagers and they have refrained from killing the lemurs, but we fear that they will lose patience if we do not make a decision. This situation is one of the reasons to perform the translocation as soon as possible.

Another reason for translocating as soon as possible and not waiting until November is the *P. simus* birth season. It is dangerous to catch and anesthetize females at the end of their pregnancy. Therefore, it is important to perform the translocation as early as possible in order to catch the females before the breeding season.

One of the main concerns about the translocation of *Prolemur simus* is "homing," the behavior of attempting to return to their native area. However, it is important to note that based on the experiments and discussions with translocation experts, the main determining factor in the animals attempting such a return seems to be the availability of resources. As a result, the season of translocation should not be a concern as special care has been taken to ensure that the new group will be satisfied with the resources available in Ranomafana National Park.

Lemurs, like all other animals, can adapt to changes in temperature as long as they are kept within the natural range of the species. Indeed, the altitude of Talatakely (900 m) is well within the known altitudinal distribution of *P. simus* is known (between 28 m and 1,600 m according to Ravaloharimanitra 2011, Wright 2008). In addition, Talatakely has been home to a group of *P. simus* for a long time. Center ValBio experts have directly observed, for 32 years, that *P. simus* was doing well in the Park, regardless of the season and temperature fluctuations (Wright 2008). Since *P. simus* resides in Ranomafana National Park, we know that the species has the necessary adaptations, thick fur and long tail, to adapt to Talatakely's temperatures. These lemurs regulate their temperature through behaviors such as sunbathing, crowding and erection of tail hair, which provides an insulating layer of air near the skin. The tail can then be used as a scarf and wrapped around the lemur for insulation of the rest of the body. Since none of these thermoregulation mechanisms are seasonal, lemurs are able to adapt quickly to changes in temperature (Chaplin 2014).

We plan to capture the group of Ivato/Karianga lemurs at an altitude of about 300 m and transport them to an altitude of about 900 m from Ranomafana. This difference in altitude change of 600 m is small considering that the natural habitat of *P. simus* extends over nearly 1600 m. This change in altitude causes only a slight change in temperature: on average, Ivato has a temperature of around 17° C while Ranomafana is around 15° C. Even were we concerned about the temperature change for the lemurs, these animals will benefit from an acclimation period of two to four weeks to adapt to the new environment, including temperature, before being released.

The main factor that would motivate the translocated animals to return to their area of origin is food availability. Thus, we have taken steps to ensure that the lemurs will be satisfied with the food available in Ranomafana and will not be inclined to leave the area. First, we conducted a survey of *Cathariostachys madagascariensis*, the preferred bamboo of P. simus, in the Talatakely area where we expect the new group of lemurs to select their new home range. The transect was carried out on plots of 10 m by 10 m over a range of 20 m by 300 m and includes the location of the acclimation cage. It is important to note that the streams crossed the transect several times and that there were extremely dense bamboo patches adjacent to the transects since bamboo is known to grow along the streams. We determined that Talatakely contains enough *Cathariostachys madagascariensis* to accommodate another 16 *P. simus*. In addition, an overabundance of this bamboo species has been observed in the Ranomafana forests outside the transect specifications and, as a result, lemurs will have abundant food even if they choose a different area for their home range.

From the observations of *P. simus* in Ivato, we know that these individuals eat not only typical bamboo species, but also rice, cassava stems, coffee and ginger stems from local farmers. These foods are not part of the natural diet of *P. simus* and bamboo from Ranomafana National Park is better for their health. At the beginning of the acclimation period, we will offer lemurs a menu of choice in addition to their natural diet based on bamboo. We hope they will simply choose not to consume crop supplements if their natural diet is abundant. However, if they express the desire for the crops, we will continue to add crops to their diet, and then slowly wean them from the cultures during the acclimation period, until they eat 100% natural food. By doing this, lemurs will be able to slowly adjust to their natural diet

rather than undergo a sudden change, which means they will be far less likely to leave Ranomafana in search of crop supplements.

A final consideration for the transfer period is the safety of the project team. Since the roads leading to Ivato are in such a bad state, the rain in November would make the roads even more dangerous to cross. It would not be good to put people and lemurs at unnecessary risk when the project can be done at a drier and therefore safer time. It would not be advisable to transfer lemurs in November in case of delay due to bad roads. The additional time of travel would be dangerous for their health and would not be in their best interest.

The translocation of *P. simus* in the Ranomafana National Park is important for the conservation of the species and so we want to make sure that everything is planned carefully, including the timing. Taking into account the livelihoods threatened by the local population, the availability of resources, the mechanisms of thermoregulation of the lemurs and the safety of the people and lemurs concerned, we believe that the transfer should take place as soon as possible and should not wait until November.

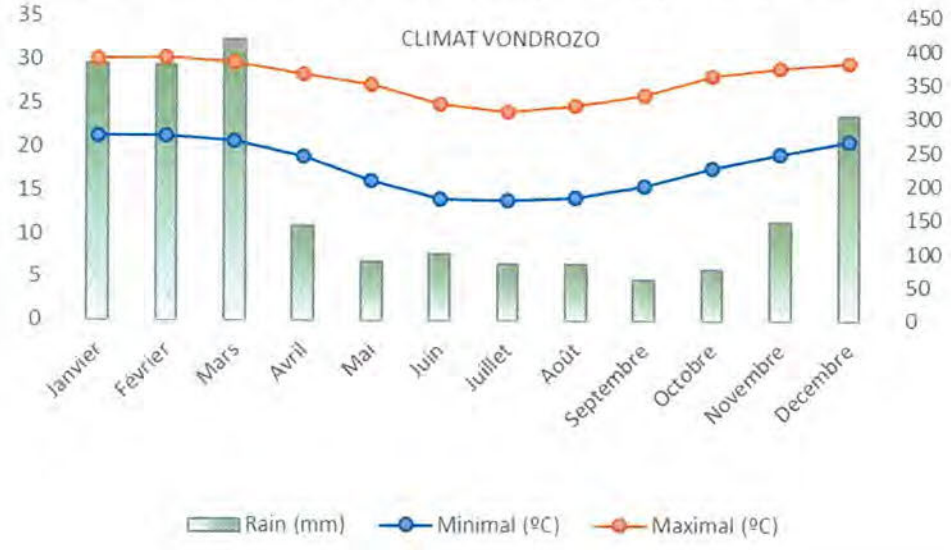
Methods

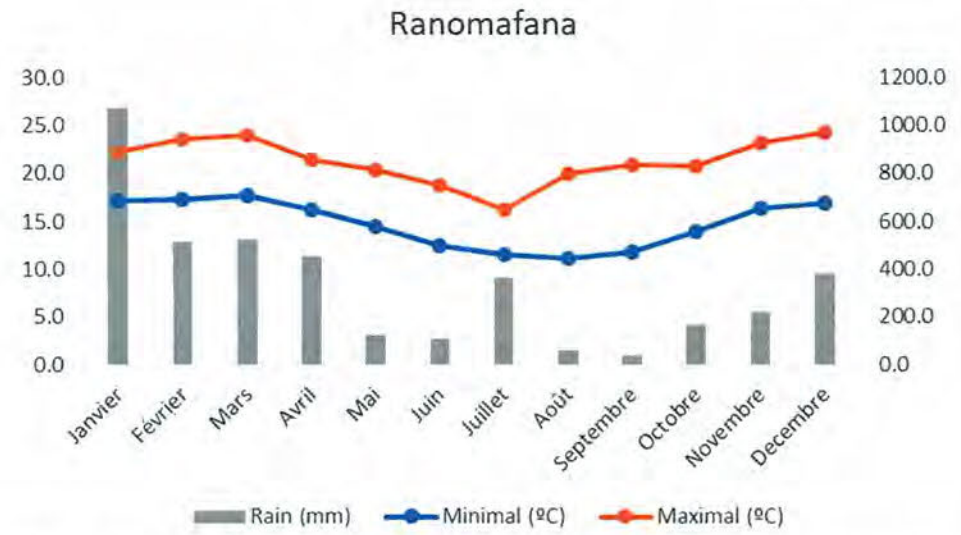
1. Study sites
   a. Capture site, Forest of Ivato. The choice of capture site for translocation is important because this site must be included in the target species' range according to the recommendation of the IUCN reintroduction specialists. It is better to choose a site close to the site of translocation to minimize stress between capture and release.

MAHASOA:	Lat: -22.422117°	Long: 47.283968°
MANGARABAKA:	Lat -22.419310°	Long: 47.281110°

This site is degraded and fragmented with three small forests of 5 ha, 7 ha and 10 ha. Bamboos are present but because of the reduced area of the forest, *Prolemur simus* travel through fields and eat cassava, sugar cane and rice.

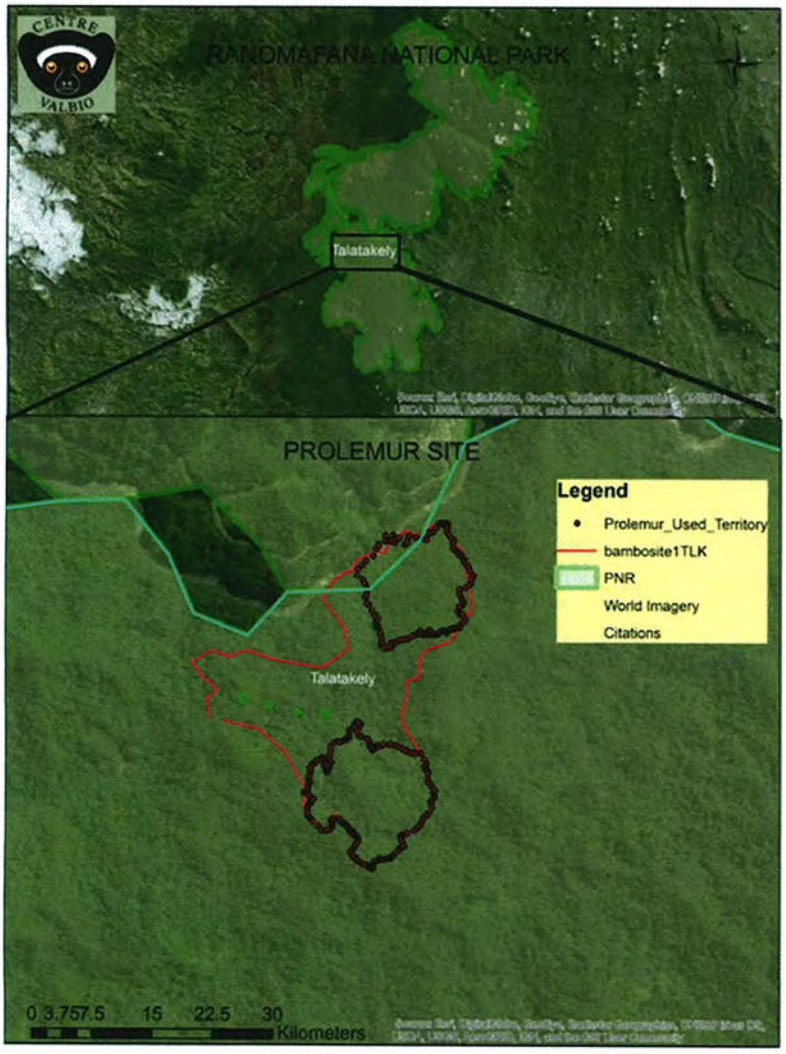
Climate at Ivato (Vondrozo) (rain and temperature) for comparison to those of Ranomafana

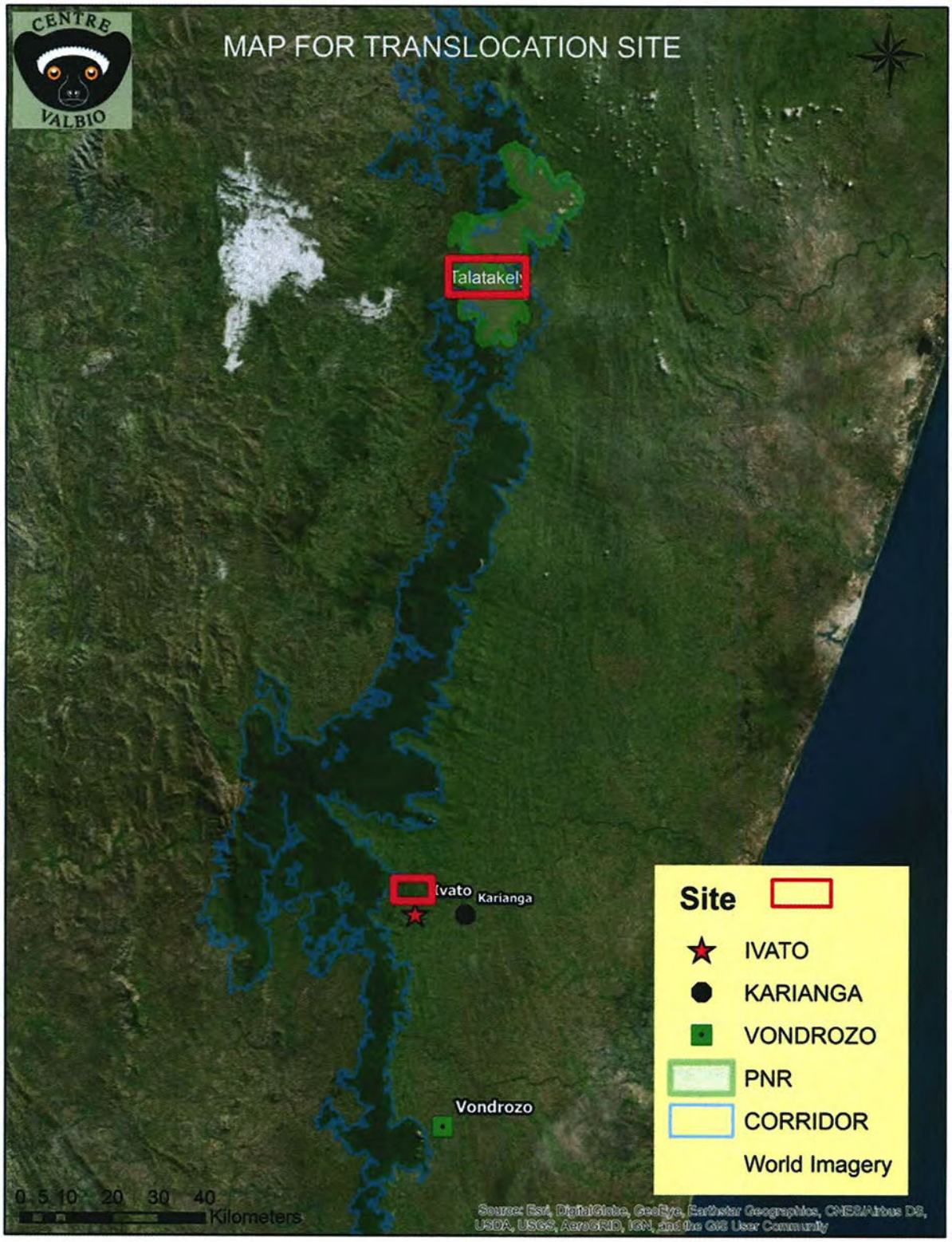




Although Ranomafana receives higher rainfall (which translates into higher bamboo growth), Ranomafana and Vondrozo have very similar temperatures. On average, Ranomafana is only a few degrees lower than Vondrozo.

b. Translocation site/release Ranomafana National Park, The forest of Ranomafana is the historic habitat of *Prolemur simus*. It has been a protected area since 1991 after the discovery of *Hapalemur aureus* there by Dr. Patricia Wright, promoter of the Ranomafana National Park Project. It is among the most visited protected areas in Madagascar according to the IUCN specialist group. According to them, a reintroduction project is possible for a site that is both a historic habitat for the animal and protected for nature conservation. This forest is notable by the sustainable management of Madagascar National Parks and partnership with Center ValBio and ICTE / MICET, which support the research, education and economic and social development projects of the local people. The release site should be typical of *Prolemur simus* microhabitat to meet their biological and ecological needs. This site should therefore be rich in *Cathariostachys madagascariensis* bamboo, the essential component of its diet. The following additional food species are complementary to its diet in the wild: *Ravenala madagascariensis, Artocarpus integrifolia*, *Ficus spp.* and *Dypsis spp.*, and *Pennisetum clandestinum* (Meier and Rumpler, 1987). The site is not close to the edge of the forest to avoid their returnto the capture site. In addition, the release site should not overlap with the territory of the resident group, especially to avoid fighting between the resident individuals and the new group. It must be remembered that the home range is the most frequented place, ie the place where the group sleeps, feeds, defends against predators, reproduces, etc. For an male adult to join a group it is better to release it nearby the existing group (the adult male and daughter) to avoid disorientation. The latter will need the help of the existing group to defend against predators and to facilitate foraging. In addition, a dominant male in its original group is likely to fit into the existing group and reproduce with the present female.


CENTRE VALBIO
RANOMAFANA NATIONAL PARK
Talatakely
PROLEMUR SITE
Legend
Prolemur_Used_Territory
bambosite1TLK
PNR
World Imagery
Citations
Talatakely
0 3.757.5 15 22.5 30
Kilometers



2. Study species, choice of captured individuals
   a. Species studied - According to the IUCN reintroduction specialists, the feasibility of reintroduction requires knowledge of the species in question, so this section discusses the biology and ecology of *Prolemur simus.* Commonly called the Greater Bamboo Lemur, this

species is the largest of the bamboo eating species. On average, its weight reaches 2.2 to 2.8 kg (Mittermeier et al., 2006) and 2.365 kg according to Glander et al. (1989). Body length can reach 40 to 42 cm with a long tail of 45 to 48 cm (Mittermeier et al., 2006). The body is olive brown with grey-brown top of the head and back, the tail is the same color as the body with a black-brown tip; head, neck, shoulders and upper arms are olive brown. Its face is broad and dull with a short, broad snout (Mittermeier et al., 2006). There is no sexual dimorphism (Tan, 1999). It is a cathemeral species and lives in groups of 2 to 12 individuals, up to 30 individuals (Rowe, 1996). Groups consist of multiple adult females and their young, with one or two adult reproductive males. The birthing period is in November (Tan, 2000). In the Ranomafana National Park, *Prolemur simus* lives in sympatry with *Hapalemur griseus* and *Hapalemur aureus*. Among the six species of bamboo that exist in Ranomafana National Park, four are consumed by *Hapalemur*: *Cathariostachys madagascariensis* (Volohosy), *Cephalostachyum perrieri (*Tsimbolovolo lavalava ravina), *Cephalostachyum viguieri* (Tsimbolovolo boribory ravina); *Poecilostachys festucaceus (*Vilon'ala). The consumed parts of each species vary according to the month and the season. In addition to bamboo, they eat fruits, leaves and petioles from other plants. It should be noted that sympatry is regulated by the separation of ecological niches.

b. Individuals captured for translocation – The individuals captured will complement the resident group (adult male and daughter). As we know that *P. simus* lives in large groups, at least 2 males and 4 females are necessary for group viability, but more is better.
    1. One of the new males should be the dominant male in the original group to facilitate acceptance by the resident female
    2. There should be at least six other females in the group
    3. A dominant male-female pair from its original group is also proposed to reduce stress and avoid disorientation and predation
    4. A total of at least 15 individuals is recommended, three males and 12 females would be ideal for the success of this project

Study before reintroduction

Description of *Prolemur simus* at Ivato (2008-2019)

In 1997, during Steig Johnson's expedition, the DVB team and Dr. Patricia Wright first saw *Prolemur simus* in Ivato, an area just north of Vondrozo and on kilometer from the CoFav Corridor. This region has forest fragments of 2 to 10 ha. In 2008, a CVB team led by Eileen Larney and Rachel Jacobs returned to Ivato and observed a group of more than 30 individuals. In 2009 and 2010, the CVB team, including Patricia Wright, created tree nurseries and taught reforestation techniques, as well as *Prolemur simus*' behavioral ecology data collection techniques to members of the local community. The guides continued to take notes on feeding and behavior of newborns from 2008 to 2010. The CVB team made annual trips to Ivato to train the local population on data collection. In 2014, the number of *P. simus* individuals had increased to 71. In 2016, we continued to teach the local community the collection of *Prolemur simus* data, but we found problems with tavy and trapping of the lemurs. Much of the lemur territory has been destroyed and replaced by crops. In March and April 2018, 5 fokontany convened a large meeting to learn about the problem of endangered lemurs and decided to give CVB permission to manage the site and the lemurs. In December 2018, the Ivato community wrote an official letter to CVB regarding their collaboration with CVB for the conservation of lemurs. Now, the population of Prolemur simus exceeds one hundred individuals and lemurs sometimes eat cassava, rice, jackfruits, coffee, sugar cane and other crops. By transferring a group of 15 lemurs, we will relieve

some of the pressure exerted by these lemurs in this area, so that the remaining individuals can be healthier.

Prolemur simus nutrition: Botanical studies in Ranomafana National Park

To meet the nutritional needs of *Prolemus simus* from Ivato, we called in an expert in nutrition, Amanda DuBour, and took steps to ensure that the animals transferred would have enough food in Talatakely, in Ranomafana National Park. First, we conducted a study of *Cathariostachys madagascariensis*, *P. simus*' preferred bamboo species, throughout the Talatakely region, where we expect the group of transferred lemurs to establish their home range. According to Chia Tan (1999), the normal diet of healthy *P. simus* in primary forest habitat is 95% *C. madagascariensis*. The botanical transect was carried out on a 20 m by 300 m plot in Talatakely and includes the location of the *P. simus* enclosure. Many streams cross the transect and bamboo thrives along streams. Dense bamboo plots also exist near the transect. We have determined that Talatakely contains sufficient *C. madagascariensis* to accommodate another 16 *P. simus*. In addition, an abundance of this bamboo species has been observed in the Ranomafana forest outside the transect boundaries and, as a result, the lemurs will have sufficient food.

Due to the slash-and-burn culture surrounding the Ivato forest fragment where the lemurs live, these individuals are not only acclimate to the consumption of bamboo species, but also to other foods such as rice, cassava stalks, coffee and ginger stalks (longoza) from local farmers, threatening the livelihoods of local people and pushing them to demand the removal of lemurs. These foods are not part of the natural diet of *P. simus* and RNP bamboo is better for their health. According to the data collected in Ivato over a period of three years from 2008 to 2010, we know that the diet of *P. simus* in Ivato is mainly composed of bamboo. In the three years of data collection, about 74% of the lemur diet was composed of bamboo volojatsy (*Valiha diffusa*). The preferred *P. simus* bamboo species (*C. madagascariensis*) is not available in Ivato forest fragments. However, the pattern of a predominantly bamboo diet in *P. simus* d'Ivato is a good sign that they will adapt well to bamboos of Ranomafana where there are many species of bamboo available for lemurs. The data collected from 2008 to 2010 demonstrate a high degree of flexibility in the diet of lemurs. In fact, during these three years, it was observed that lemurs consumed plants of eight different species. Although *V. diffusa* remains the largest part of the diet over the three years, the food constituting the rest of the diet varies considerably. The eating habits highlighted by this population demonstrate a degree of flexibility that indicates that new lemurs will adapt well to the Ranomafana diet. Some of these additional species, including longoza (*Aframomum angustifolium*) and voatrotrok'ala (*Clidemia hirta*), which accounted for about 37% and 13% of their diet in 2008 respectively, are also abundantly found in Ranomafana, offering more options familiar to lemurs after translocation.

To fully understand the distribution of food for these lemurs, MNP and ValBio Center staff conducted surveys and selected sites for botanical transects. The results of this bamboo distribution survey (*Cathiostachyium vigueri)* carried out in 2019 show that a sufficient quantity of bamboo still exists in the group's territory in the Park. A study of the place to put the *P. simus* in the Park was carried out by agents of MNP and ValBio Center. A temporary enclosure 15m x 4m x 15m high made by fences will be built in the park to accustom the captured *P. simus.* This enclosure will be established in a flat spot between the Sakaroa and Mariavaratra rivers, far away from the tourists on the P trail. The advantages of this location are the abundance of bamboos and the absence of big trees that would need to be cut during construction of the enclosure. This location is easily accessible from the main trails but far from the places frequented by tourists.

Approximately 50 faecal samples will be collected to analyze existing parasite species and their prevalence. Individuals will be monitored to decide which will be chosen for the translocation. At the same time, the CVB technical team will follow the *Prolemur simus* group in the Ranomafana National Park.

Capture of animals outside of the Park

At least 15 individuals from a group in the fragmented Ivato forest will be capture, examined by a veterinarian to determine health status, and marked with a radio collar and translocated to Ranomafana. We will be careful not to catch pregnant animals. We know that *Prolemur simus* is not monogamous and forms a large group that can reach 11 - 38 individuals with a territory up to 50 ha (Wright et al., 2008, Louis et al., 2012). We also know that reproduction does not occur in a small group and competition between males increases reproduction (Wright, et al, 2008, Roullet, 2012).

a. Capture and physical exam
   Capture method and assessment protocol are as detailed by Junge et al. (2008). Each individual will be captured using a dart gun with Telazol at 10 mg/kg (Dan-Inject Model MJ, Dan-Inject, Knoxville, TN) (Type C Disposable Dart, Tire-Dart, Williamsport, PA). A physical exam and sampling (tissue, blood, etc.) will be conducted while the animal is anesthetized. For the physical examination, the following parameters are recorded: weight, temperature, pulse rate, respiration rate, time of capture (hour and minute), tissue removed (number and size), blood collected, total dose of anesthesia used, measurements of the fore and hind limbs, microchip reference number, and canine measurements.
b. Tissue collection
   For genetic analysis, four 2.0-mm biopsies will be taken from each animal. These samples will be stored in room temperature tubes (Longmire et al, 1992). In addition, 1.0 cc of blood per kilogram of body weight will also be collected for biomedical analysis. A microchip will be implanted subcutaneously between the shoulder blades of the animal for permanent identification. A low-frequency radio collar will be placed to follow the animal after release. The frequency of the collar of each animal is verified on site with the use of a receiving antenna.

   Dr. Wright, a team of technicians from Centre ValBio, Malagasy veterinarians and American and Malagasy students will embark on an expedition to the remote village of Ivato. The team will camp at Ivato for two weeks, observe lemur behavior and collect faecal samples to determine the presence of parasites to select a group of 15 lemurs that would have the best chance of success in translocation. We will bring slides and a microscope for Ezzeldin Enan to analyze the faecal samples of all potential lemurs for transfer so that they can be evaluated and treated for parasites. When we are sure that the Ivato individuals are in good health, we will capture them with anesthetic darts with Telazol in accordance with the ValBio Center Lemurs Anesthesia Protocol and the capture team will use nets to ensure that the lemurs are safe during capture. The CVB capture team and veterinarians will check their health. Bekah Weatherington and the veterinarian will monitor anesthesia, complete physical exams (including temperature, heart rate and breathing), blood tests, and collar placement. Blood will be taken from sedated lemurs and placed in heparinized blood tubes and blood tubes containing ethylene diamine tetra-acetic acid (EDTA). The heparinized blood will be put in a centrifuge and the plasma will be pipetted and transferred to a cryotube which will be placed in liquid nitrogen for storage. We will also have hematocrit tubes, a clay sealing tray, a packed cell volume reading card (PCV), a refractometer and a portable centrifuge. With these devices, managers could start analyzing

field samples. However, some of the samples will be transported to Centre ValBio laboratories and/or exported to the USA for further analysis. The forms for the collected data will be presented as attachments.

c. Transfer to Ranomafana and acclimatization/habituation: Each animal is placed in a plastic carrier where it will receive bamboo for the duration of the trip. The team will monitor the animals during their transfer to Ranomafana where they will be placed temporarily in a pen to allow acclimatization and monitoring of their health. Indeed, with the approval of the officials of Madagascar National Parks, the animals will be transferred to an enclosure inside the Park to begin the habituation. The team will monitor the health of the lemurs during their acclimatization/habituation to make sure they eat well and behave normally. They will be fed daily in the pen until they are released in Ranomafana National Park.

d. Release and following:
All captured animals will have collars and the CVB team of technicians and researchers will follow resident and released lemurs every day for at least a month. All feeding behavior, aggression, reciprocal grooming and rest will be recorded. If there are problems, the capture team will be available to recapture them for a checkup.

The translocated animals will be released when we are sure that the resident animals are approaching the cage and we can observe the interactions.

Between 14 and 30 days, when the animals are used to the cage, an animal vocalization recording from the Talatakely territory will be playe in the morning. Observers will record all the behaviors of these animals in response to this vocalization. Resident individuals will also be monitored and their response to the vocalization will also be recorded.

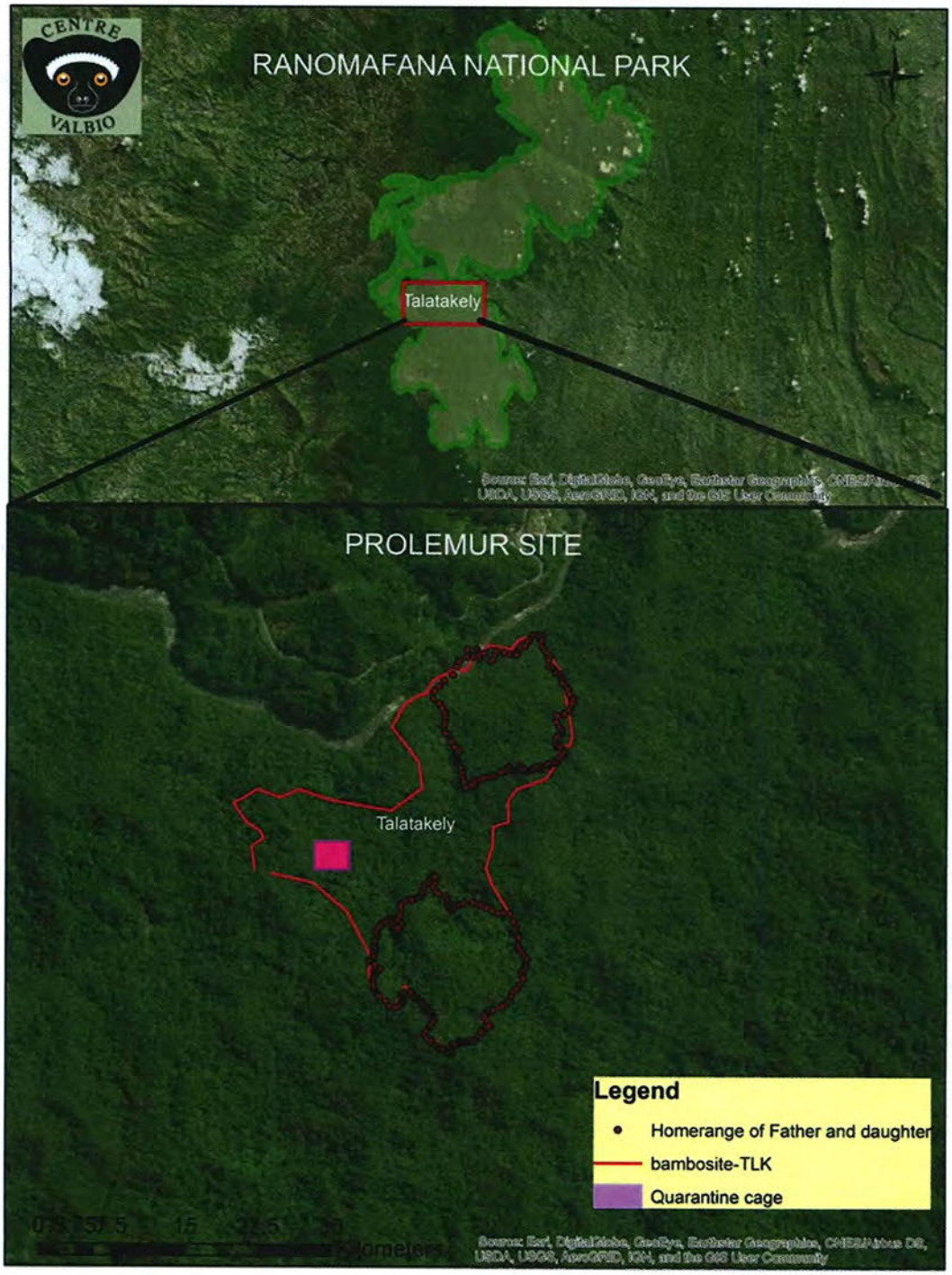
We hope that the resident Talatakely group will eventually be attracted to vocalisation from the translocated individuals and will come to the cage and see the new individuals. This two-week quarantine period will give both resident and transferred groups an opportunity to meet and interact before the release of the new group.

Once released, we will follow their movements using the tracking collars. At the same time, we will also follow the resident group.

Description and location of the enclosure

The enclosure will be constructed in Talatakely, in Ranomafana National Park, outside the home range of both RNP *Prolemur simus* and in the bamboo cover area. The enclosure will measure 15 m by 15 m and 4 m high. It will be built by wood fences. The enclosure will be located in a dense bamboo location and away from the Talatakely trails so that the lemurs are not disturbed by tourists.

Quarantine: Animals should be released when their health status is verified and confirmed by the veterinarian. Indeed, the release can be done when the animal is acclimatized to the surroundings and predators. Animals should be released as soon as possible in previously studied areas. Indeed, *Prolemur simus* is very sensitive to different stresses and keeping the animal in a pen for quarantine would only increase their stress, according to IUCN guidelines for good practice, the lemur can remain at a minimum 14 days in the cage and up to 30 days. They will be fed twice a day.



Monitoring will therefore be done using radio collars and adopting the Altman (1974) method. During the observations, the general activities of the animal are recorded every five minutes to know the locomotion, movement, the food (plant species, part consumed, etc) and others (communication, affiliation, mating, aggression, etc.). The coordinates of travel are recorded to estimate territory size and identify feeding and sleeping sites.

Competing or predatory species encountered are also noted (species name, geographic coordinates of meeting places) to estimate predator pressure.

This ecological monitoring is done at least 5 days a week until the group's life stabilizes, i.e. when the group starts to have a defined territory or join a local group.

When the life of the released groups is stable with a more or less defined territory, we will proceed to study its new microhabitat to know its structure and botanical composition. Sampling will be done in 4 botanical blocks of 20mx50m at different altitudes (ridge, slope, bottom). In each botanical plot the name and family of the woody species are identified plus recording trees > 5 cm DBH (diameter at breast height), the bamboo species, and the tree canopy volume and abiotic characteristics of trees > 5 cm DBH.

A reintroduction assessment will be carried out at the end of the first year of follow-up. The genetic analyzes of the collected data will be carried out as soon as possible and the results will also be used for the evaluation of reintroduction.

We will establish a post-release education system on the reintroduction project to avoid misunderstandings with the local residents.

The success of this project will be measured by the following criteria:
1) Individuals introduced into this group of Talatakely are accepted into the resident group.
2) Infants will be born in the group within three years of reintroduction
3) The remaining Ivato fragment group will not be affected by the reduced number of individuals

Long-term follow-up:

Regular and ongoing animal monitoring in the long term will be carried out by Centre ValBio and Madagascar National Parks, Ranomafana. Monitoring will be carried out according to the standard ecological monitoring protocols, at least once a year for a period of 2 - 3 months, preferably during the breeding season until birth.

The collars will be replaced after 18 months and an assessment of the health of the animals will be made during this collar replacement.

For long-term follow-up, each institution will have clearly defined roles and responsibilities:
Centre ValBio, with Madagascar National Parks, will provide technical and scientific coordination and follow-up for the conservation and research component.

Madagascar National Parks, Ranomafana, will facilitate obtaining the necessary permits and approaches for the follow-up with the Ministry of the Environment and Sustainable Development.

Expenses during these follow-ups will be discussed and shared among the partners.

List of participants:

- Patricia Wright – Principal Investigator
- Bekah Weatherington – Sampling supervision
- Ezzeldin Enan – Sampling supervision
- Fidy Rasambainarivo- Veterinarian
- Haja Rakotondrainibe - Veterinarian
- Jake Krauss – Student assistant for behavioral follows

- Amanda Du Bour – Student nutritional assistant
- Ryan Rothman - Student nutritional assistant
- Lianne Woudstra - Student nutritional assistant
- Thomas Kelly - Student assistant for behavioral follows
- Tobias Gräßle - Veterinarian
- Sina Feyer - Veterinarian
- Heninkaja Rasoaviarimanana – Student nutritional assistant
- 2 représentants MNP – supervision
- Représentants MEDD – supervision
- Pascal Rabeson - supervision
- Velotsara Jean Baptiste – Darting technician
- Dina Andrianoely – Research technician
- Georges Razafindrakoto - Darting technician
- Mamitina Velonabison - Darting technician
- Georges Rene Rakotonirina – Behavioral technician
- Remi Rakotovao – Behavioral technician
- Dominique Razafindraibe - Behavioral technician
- Randrianasolo Laurent – Behavioral technician
- Zakamanana Francois – Behavioral technician

Conclusions and recommendations

In conclusion, all the conditions for reintroduction are fulfilled and it is evident that the translocation of a few *P. simus* individuals from the Ivato forest and their reintroduction to the Talatakely forest to save the remaining population of two individuals is very important for the conservation of this species and the future of tourism at Ranomafana. After this reintroduction, genetic diversity will be ensured for the continued viability of this protected population. We are convinced of the success of this reintroduction project and hope that this example will be a model for the reintroduction of a species at risk both at national and international level.

References

LEMURS HANDLING SHEET (VALBIO CENTER _ JULY 2019)			
DATE:	RESEARCHERS NAME:		
LEMURS IDENTIFICATION			
ID		MICROCHIP	
SPECIES		SEX	
GROUPE		AGE EST	
INDIVIDUAL AFFILIATION		NEW RADIO	
DISTINGUISHING MARK		NEW NYLON	
IMMOBILIZATION INFO			
DATE/HOUR		ZONE	
CAPTURE METHOD		GPS	
DRUG		DOSE	

PHYSICAL EXAM				
	NOR	ABN	COMMENTS	
BODY CONDITION			OVERWEIGHT / GOOD /	
ORAL CAVITY			BLOOD / OBSTRUCTION	
MUCOUS COLOR/CRT				
TEETH			MISSING / BROKEN	
MOLAR WEAR			NONE / MILD / MODERATE / SEVERE	
TOOTH COMBWEAR			NONE / MILD / MODERATE / SEVERE	
EYES			DULL / DISCHARGE / INFLAMMED / CLOSED	
EARS			INJURED / DISCHARGE / PARASITES / DEBRIS /	
NOSE			DISCHARGE / DRY / SORES / LACERATION	
HAIRCOAT			THINNING / UN-GROOMED	
SKIN			SORES / WOUNDS	
TICKS / LOCATION:			FEW / MOD / MANY	
MITES / LOCATION:			FEW / MOD / MANY	
LICE / LOCATION			FEW / MOD / MANY	
FLY /LOCATION			FEW / MOD / MANY	
ABDOMINAL PALP				
LYMPH NODES				

MUSCULOSKELETAL			LIMPING / DECREASED	
CARDIO			MURMUR /ARRHYTHMIA	
RESP			CRACKLES / WHEEZES	
NIPPLES				
GENITAL/URINARY:				
SC FLUIDS				

SAMPLE COLLECTION				
	Y	N	QUANTITY	COMMENT
BLOOD (EDTA-purple top)				
BLOOD(heparin-greentop)				
DENTAL PHOTOS				
LESION PHOTOS				
TISSUE SAMPLE				
URINE (red top)				
BREATHE				
SWABS				SCENT GLANDS/GENITAL/RECTAL/OCULAR/NASAL/ORAL
ECTOPARASITES (ethanol)				TICKS / MITES / LICE / FLIES
FECES(formalinandPVA)				
HAIR				
ID PHOTOS				
FINGERPRINTS				

MEASUREMENTS			
GENERAL BODY			
Weight (Kg)		Biceps circ. (cm)	
Head crown(cm)		Chest circ. (cm)	
Body length (cm)		Thigh circ. (cm)	
Tail length (cm)		Nipples (cm)	
FORELIMB (length)		HINDLLIMB (length)	
Thumb (cm)		Thumb (cm)	
Longest digit (cm)		Longestdigit (cm)	
Hand (cm)		Foot (cm)	
Ulna/Radius ((cm)		Tibia (cm)	
Humérus (cm)		Fémur (cm)	
CANINE		MUZZLE	
Upper (mm)		Length (mm)	
Lower (mm)		Width (mm)	
EAR			
Length (mm)		Width (mm)	
TESTES			

Right length (mm)		Left length (mm)	
Right width (mm)		Left width (mm)	
GENERAL COMMENTS			

ANESTHESIA				
ANAESTHESIC SUPPLEMENT			RECOVERY	
TIME	DRUG	DOSE	TIME	COMMENT

VITAL SIGNS			
TIME	HEART RATE (BPM)	RESP RATE (BPM)	TEMP (F / C)

Blood

Green tube:

PCV (%)_____ Glucose _____

TP _____ Plasma appearance _____

Purple tube: CBC

White Blood Cell Estimate: on 40X or 50X on the monolayer

Field 1	Field 2	Field 3	Field 4	Field 5	Average

Avg. X obj^2= _____

White Blood Cell Differential: 100X oil immersion on the monolayer

	Cell Count	Differential Count (%)	Absolute count (x tWBC)		Cell Count	Differential Count (%)	Absolute count (x tWBC)
Segmented neutrophils				Monocytes			
Band neutrophils				Basophils			
Lymphocytes				Other			
Eosinophils				Total	100	-	-

Platelet smear estimate: 100X oil immersion on the monolayer

Field 1	Field 2	Field 3	Field 4	Field 5	Average

Avg X 15,000 = _____ (180-225,000/uL)

Azostix (BUN):_____

Hemoparasites:

WBC morphology: RBC morphology:

Urine

Color: _____ Clarity: _____ Refractometer- Urine Specific Gravity: _____

Dipstick results:

Leukocytes	Nitrites	Urobilinogen	Protein	pH	Blood	USG	Ketones	Bilirubin	Glu

Sediment Exam:

Bloodwork Protocol for Lemur Translocation- Bekah Weatherington

Purple top tube (EDTA anticoagulant): yields plasma, contains fibrinogen and clotting factors

- Mix well using inversion
- Promptly make 2 blood smears
- Let air dry
- Dip quick stain

- 5 dips in each stain
- Rinse with water

- Let air dry
- Examine under microscope
  - CBC
    - WBC estimate
      - Use 50X on the monolayer
      - Avg in 10 fields X 2,500 (or the objective squared, ie multiply by 1600 if on 40X)
    - WBC differential
      - Use 40X or 100X oil immersion
      - Count 100 total
    - Platelet estimate
      - Use 100X
      - Avg in 5 fields X 15,000
      - Tip: normally see 12-15 platelets/hpf, equating 180-225,000 platelets/microL
    - Note WBC and RBC morphology
  - Hemoparasites

Green top tube (heparin anticoagulant): yields plasma

- PCV and TP
  - Load 2 hematocrit tubes
  - Spin down- 5 min on hematocrit centrifuge
  - Measure PCV using reader card
  - Break tube and measure TP on refractometer
- Glucose
  - Place strip in glucometer
  - Touch end to drop of blood (follow glucometer manual)

- CHEM panel- if red top tubes unavailable, only if have the ability to freeze (liquid nitrogen)
  - Need to fill 2 green microtainer tubes
  - Centrifuge
  - Pipette off 1ml plasma
  - Freeze to store until able to run CHEM panel in lab

Red top tube (no anticoagulant, clotted blood): yields serum, no fibrinogen, some clotting factors

- CHEM panel- red top tube ideal, only if have the ability to freeze (liquid nitrogen)
  - Allow one hour to clot
  - Centrifuge
  - Pipette off 1ml serum into clean tube
  - Freeze to store until able to run CHEM panel in lab

Protocol for Sample Collection During Field Immobilization

We are following the collection protocol recommended in Gillespie et al 2008. These samples will be collected during field immobilization.

- Each
  - Any observed abnormalities
  - Examination of the oral cavity, eyes, and ears
  - Auscultation of heart and lungs
  - Recording of body temperature and respiratory rate
- External measurements including:
  - Weight
  - Body length
  - Tail length
  - Girth (At widest point)
  - Neck circumference
  - Foreleg / Forefoot length
  - Hindleg / Hindfoot length
- The dentition of each animal will be digitally photographed
- The Following samples will also be collected as recommended in Gillespie et al. 2008:
  - Blood: 10–20 ml, not to exceed 1% of body weight, should be collected from the femoral vein after disinfecting the site with alcohol. Appropriate sized needles must be selected to prevent trauma and venipuncture site must be monitored briefly for hematoma formation. Today, collection of EDTA blood is recommended since this allows analyses of whole blood, plasma and "Buffy Coat". A drop of the blood collected should be used for thin and thick smears, then the collection tube can either be centrifuged to separate plasma from cells or (if no centrifuge is available) EDTA tubes can be stored standing until the plasma has separated from the cell-rich fraction. Once separation has occurred, plasma and cells should be stored separately frozen (or if not possible dried).
  - Hair: Several pinches of hair pulled from the base of the tail should be collected as a source of genetic material and for detection of integumentary pathogens. A glove or instrument must be used to avoid contamination with human tissue.
  - Mouth swabs: Inside of the cheeks should be swabbed for detection of oral pathogens.
  - Tracheal swabs: Swabs of the trachea should be collected for detection of respiratory pathogens. This is a challenging procedure for many species and requires some level of expertise. This procedure also presents a small but real risk of an animal bite, even in an anesthetized animal, due to jaw reflexes.
- Animal will receive a complete physical examination including:
  - Penile/vaginal swabs: Swabs of the penis or vagina should be collected for detection of sexually transmit- ted pathogens.
  - Rectal/fecal samples: Rectal swabs and fecal samples should be collected for detection of gastrointestinal pathogens.
  - Ocular swabs: An eye should be swabbed for the detection of ocular pathogens. This should be done with care to avoid corneal trauma from abrasion.
  - Nasal swabs: Swabs of the nose should be collected for detection of respiratory pathogens.
  - Ectoparasites: The hair and skin of the animals should be examined with fine-toothed combs. Any ectoparasites (e.g. lice, ticks, fleas) thus obtained should be saved for identification in 70% ethanol.
- These samples will be refrigerated, stored in RNALater, or dehydrated for future pathogen analysis

Collection of Fecal Samples for Gastrointestinal Parasite Analysis

- Each Fecal sample will be split into 10% Buffered Formalin for Helminths and Polyvinyl Alcohol for Protozoa

- Fecal samples will be examined macroscopically for consistency, blood, mucus, or visible larvae. Observed helminths will be preserved for identification
    - Nematodes will be washed 3 times with 1% saline solution. They will then be transferred to hot 70% Ethanol or 5% formalin (70-80°C). After cooling, they will then be transferred to a cool solution of the same kind.
    - Cestodes (Tapeworms) will be fixed in 5-10% formalin between two pieces of glass. They can also be dipped repeatedly while suspended with forceps to prevent contraction
    - Trematodes (Flukes) will be washed with 1% saline while being vigorously shaken. Saline will then be replaced by 5-10% formalin while still shaking.
- Using a wooden applicator stick, a 2g fecal sample will be collected from inside the bolus to prevent contaminants and placed in a 15mL centrifuge tube. (6g for 50mL tubes). The tubes will then be vigorously shaken.
- Tubes will be properly labeled with the Individual's ID number. Notations will be made containing observed Age, Sex, GPS coordinate of sample, Date & Time of collection.

The table below has an overview of the information collected per sample. Daily measurements of temperature, humidity, precipitation, etc. will be taken and noted during sample collection.

- If possible, samples will be stored in a fridge 4°C. If not, they will be kept in a cool dark place until laboratory analysis.
- If possible, samples will be duplicated and placed in RNA later for molecular confirmation of parasite identities.

Sample Number	Example ID# 1234	1	2	3
Preservative F=10% buffered formalin P=Polyvinyl Alchohol E=Ethanol	F			
Name	Prolemur bekah			
Date	06/03/2019			
Time of Defecation	14:23			
Time of Sample Collection	14:24			
GPS Coordinates of Sample collection	-21.237848, 47.435306			
Age	3			
Sex	Female			
Macro-observation	Blood in feces; Nematode ID:1234; Hair in feces			
Consistency of Feces H=Hard; M=Medium; S=Soft; F=Fluid	S			
Comments	Lactating; Sneezing; Lethargic etc.			
Stored at R= Room temp; F=4°C Fridge	R			
Collected By	Ezzeldin Enan			
Date of Laboratory Analysis	06/04/2019			

Fecal Floatation

- Fecal Floatation will be done using Sheathers sucrose solution with a specific gravity of 1.27 as well as Zinc Sulfate ($ZnSO_4$) with a specific gravity of 1.18

- 1-2g of feces will be placed in a 15mL centrifuge tube and ~10mL distilled water will be added. The mixture will then be homogenized using a wooden applicator stick.
- They will then be centrifuged at 1800 rpm for 10min before pouring off the supernatant and resuspending it in Sheathers sucrose solution or zinc sulfate
- The additional solution will be added to the tube until an inverse meniscus and a cover slide will be placed on top and centrifuged for another 10 minutes at 1800 rpm
- After centrifugation, the coverslips were placed on prelabeled microscope slides with the Sample ID number.
- Slides will then be examined systematically for parasites. Lugol's Iodine Solution will be used as a simple stain to facilitate identification.
- Parasite egg, larvae, and cyst morphology will be measured using a calibrated ocular micrometer
- Representatives will be Photographed

Fecal Sedimentation

- Fecal samples will be suspended in 40 mL of diluted soapy water (Non-toxic biodegradable soap)
- The suspension will be filtered through a double layer of gauze presoaked in the sedimentation solution. The remaining pellet and gauze will be washed down using an additional ~5-10mL of sedimentation solution, into a centrifuge tube.
- After centrifuging for 10 minutes, the supernatant will be decanted and the sediment resuspended in the sedimentation solution. This will be repeated until the supernatant is clear.
- A few drops of the sediment will then be placed on a pre labeled microscope slide and covered with two coverslips.
- Slides will then be examined systematically for parasites. Lugol's Iodine Solution will be used as a simple stain to facilitate identification.
- Parasite egg, larvae, and cyst morphology will be measured using a calibrated ocular micrometer
- Representatives will be Photographed

Opportunistic Necropsy

- A rectal/fecal sample will be collected for later analysis
- After ligating and removing the stomach and intestines, string will be used to tie the stomach, small intestine, large intestine, and colon.
- Each section will be cut open and contents collected and washed separately.
- The walls of the stomach and intestine will be thoroughly washed and any larvae collected.
- The wash will be filtered through 0.15mm aperture for recovering adult worms, and then 0.038mm for recovering immature worms. The screen will then be repeatedly washed until it runs clear.
- The surface of stomach, intestines etc. will then be carefully examined with a dissecting microscope for parasites that remain attached.

Trichrome Stain Protocol for Parasitology PVA-Fixed Specimen

- Allow specimen to fix in LV-PVA (Low-Viscosity Polyvinyl Alcohol) for at least 30 minutes. Mix thoroughly with applicator sticks.
- Pour a small amount of the LV-PVA fixed material onto a paper towel, and stand for 3 minutes to absorb excess PVA.
- Using an applicator stick, apply (Do not smear) some of the material from the paper towel onto microscope slides.
- Dry the slides overnight at room temperature
    - Or at 37°C in an incubator for several hours
    - Or on a slide warmer for several hours

Accelerated drying is not recommended due to morphology distortions ○ Do not proceed until slides are completely dry. Once the slides have dried completely, proceed with the following staining protocol.

70% Ethanol Plus Iodine Stock Solution Procedure

1. Prepare a stock solution by adding iodine crystals to 70% alcohol until a dark solution is obtained (1-2g/100mL)
2. To use, Dilute the stock solution with 70% alcohol until a dark red-brown or strong tea color is obtained

90% Ethanol Plus Acetic Acid

1. Mix the Following:
    1. 90% Ethanol (99.5mL)
    2. Acetic acid (Glacial) (0.5mL)

Stain Procedure

70% Ethanol plus Iodine	5-10 minutes
70% Ethanol	5 minutes
10% Ethanol	3 minutes
Trichrome Stain	10 minutes
90% Ethanol plus acetic acid (Drain slides immediately and proceed)	1-3 seconds
100% Ethanol	Dip 2-3 times
100% Ethanol	3 minutes
100% Ethanol	3 minutes
Xylene	5-10 minutes
Xylene	5-10 minutes
Mount with a coverslip and Mounting Medium	

Results:

Protozoan trophozoites and cysts will be readily seen. *Entamoeba histolytica* w ill have a blue-green cytoplasm and cysts will also appear blue-green. *Entamoeba Coli* cysts will appear blue-green with a purple tint. Helminth eggs and larvae (wet smears from concentration are recommended) retain stain

and will appear red-purple. Yeast may be identified and will appear green. Human cells such as RBC's will appear red. Karyosomes of nuclei, chromatoid bodies, and chromatin material appear red-purple.

Collecting Swabs of Scent Glands

Items needed:

- Small container or Ziploc bag (to hold sterile materials)
- Cotton-swabs (sterile)
- 15mL falcon tubes (sterile)
- Scissors (clean)
- Alcohol wipes (to wipe down scissors before every use)
- Examination gloves
- Cooler with ice or ice packs (to keep samples cool until freezing)

Procedure:

1. Put on examination gloves for sampling. If gloves become contaminated, you should replace them.
2. Use an alcohol wipe to sterilize scissors (wipe down the blades of the scissors).
3. Cut a sterile cotton swab in half.
4. Carefully remove the sterile cotton swab from the packaging by pulling out the handle.
5. Obtain sample by rubbing the swab across the glandular region three times.
6. Open a sterile falcon tube and immediately place the swab (tip in first) into the tube. Close the lid of the tube immediately.
7. Place the sample into the cooler and transport it back to lab.
8. Store the samples in a freezer (ideally -80 C).

Whenever possible, it helps if two people work together to prepare the materials and obtain the sample. Be sure to always use gloves to ensure that materials are free of DNA contamination.

***Prolemur simus* Breath Sampling Protocol**

To sample volatile organic compounds (VOCs) that are exhalants from bamboo lemurs, we will use a method developed (Zohdy, Starkey, Blagburn patent pending) which has been optimized for the detection of breath compounds only and not environmental surroundings.

Equipment:

- Teflon coated tubing
- Modified gas pump with in/out valve
- Modified coated flexfoil bags with sealable septum -Sharpie for labeling
- Scissors
- Rubber gloves

Sampling:

- The sampler should put on a fresh pair of rubber/nitrile gloves per sample bag -Cut two 3-4 inch long pieces of tubing
- Attach one to a sampling bag and "out" valve on pump
- Attach the other piece of tubing to the "in" valve
- Turn on pump and hold tubing connected to "in" valve ~2 inches away from the lemur's nostrils to capture exhalant. Keep pump on for 1-2 minutes or until bag is 1⁄2 to 3⁄4 full
- Turn off pump
- Close valve attached to sample bag until sealed. Make sure there is no leak and that it is sealed shut
- Label bag with date, location (GPS coordinate), collector's name, species name, individual ID, sex, body mass, type of ectoparasites found, quantification of ectoparasites (true number or infestation level: none, low, medium, heavy)
- If possible, please collect representative ectoparasites in ethanol labeled with individual ID

As a negative control, collect a sample bag using the same methods listed above, but collect ambient air from the location in which you sampled the lemur (the forest, campsite, bamboo forest, ag site, etc.). Label the bag in the same way. This is very important for distinguishing breath VOCs from others. A negative control bag should be collected for each location where lemurs are captured.

PROPOSITION DE PROJET DE REINTRODUCTION et TRANSLOCATION DE *PROLEMUR SIMUS*

DANS LA FORET DE TALATAKELY DU PARC NATIONAL DE RANOMAFANA,

Présentée par la Mention Anthropobiologie et Développement Durable et ICTE/CVB et MNP
Représentée par Pr. Patricia Wright

patchapplewright@gmail.com

CONTEXTE

Prolemur simus, connu auparavant sous le nom de *Hapalemur simus* (Groves, 2001), est de loin le plus menacé des espèces de lémuriens à Madagascar (Ganzhorn et al., 1996/1997; Mittermeier et al., 2010 Wright, et al, 2008). Les données à partir des subfossiles connus ont montré que cette espèce était largement distribuée à Madagascar, Anjohibe et massifs d' Ankarana au Nord, les caves dans les Tsingy de Bemaraha à l'Ouest et même dans les hauts plateaux à Ampasampazimba , 25 km à l'Ouest d Antananarivo (Godfrey et al., 2004; Godfrey and Vuillaume-Randriamanantena, 1986; Simons, 1997). Les *P. simus* actuel se trouvent dans les forêts humides du Sud Est. Avant les années 70, *P. simus* était seulement connus dans deux sites, la plantation de café de Kianjavato et la forêt de Vondrozo (Meier et al., 1987; Meier and Rumpler, 1987; Petter et al., 1977; Wright et al., 1987). A la suite de quelques recherches et des destructions massives de la forêt, cette espèce a été suspectée comme éteinte (Godfrey and Vuillaume-Randriamanantena, 1986) mais les deux équipes de recherches qui sont arrivées à Madagascar en Juin 1986 ont trouvé des individus de cette espèce. Un groupe de 12 individus de *P. simus* ont été observé dans la plantation de café de Kianjavato (Wright et al., 1987), et probablement le même groupe (N = 6 individus) a été observé au même endroit plusieurs mois après (Meier et al., 1987; Meier and Rumpler, 1987). De plus, un second groupe de 11 individus a été observé dans la forêt classée de Ranomafana (Meier et al., 1987; Wright, 1988; Wright et al., 1987). Une des raisons pour mettre cette forêt sous protection, Parc National, est la présence de deux espèces rares, *Hapalemur aureus*, une nouvelle espèce, et *P. simus* (Wright, 1992; Wright and Andriamihaja, 2003).
Le rapport des recensements effectués dans le Sud Est ont confirmé que *P. simus* est gravement menacé (Wright, et al., 2008) et ce rapport a inspiré les recensements dans d'autres zones. De nouveaux groupes ont été découverts dans les sites tels que Ranomainty et Sakalava du Corridor Ankeniheny-Zahamena et la région de Vohiposa region près de Brickaville (Randriarimanana, et al, 2012, Feaniaina, et al, 2012, Anjara et al., 2012), la partie Nord Est de la forêt de Kianjavato, fragment de Vohitrarivo (Tsaratanana) et dans la partie Sud à Ivato, Karianga. (Toutefois les seuls groupes dans une aire protégée sont les groupes dans le Parc National de Ranomafana).

Hapalemur aureus et *Prolemur simus* sont les symboles du Parc National de Ranomafana. Les études à long terme sur ces animaux ont révélé qu'il y a une diminution du nombre des individus dans le groupe de *Prolemur simus* du site de Talatakely. En effet, le site de Talatakely présente quelques groupes de *Prolemur simus* mais ce groupe est le seul qui est habitué des touristes et des chercheurs. Ce groupe ne présente actuellement que deux individus (père et fille) alors qu'il comprenait plus de 10 individus au début des observations il y a une dizaine d'années (Wright et al., 2008). D'autre part, les suivis et reconnaissances aux alentours du Parc ont permis d'identifier des groupes de *Prolemur simus* dans le fragment de forêt d'Ivato. Nous avons donc pensé qu'une translocation des individus de ce groupe vers le groupe de Talatakely serait une solution pour augmenter l'effectif du groupe de *P. simus* pour ne pas avoir de problème de consanguinité. Ce projet de réintroduction étaient initié en collaboration avec les responsables de la Direction Régionale du Ministère de l'Environnement et du Développement Durable, les responsables de Madagascar National Parks et les agents et scientifiques du Centre ValBio en Septembre 2012. Quinze individus (trois male et douze femelles) du groupe à l'extérieur du Parc seront capturés et introduits parmi le groupe de Talatakely. Puisque ces groupes se trouvent à une distance considérable du groupe de Talatakely. Nous prévoyons un succès quant à l'acceptation des groupes et par la suite la reproduction.

La diminution de nombre des individus dans l'aire protégée a été discutée pendant la réunion de l'IUCN à Antananarivo en Juillet 2012, et tout le monde ont accepté que ce serait une bonne stratégie. La réussite du Projet de la fondation Aspinall dans le Corridor Ankeniheny-Zahamena (CAZ) ainsi que celui de Brickaville qui consiste

à la protection de nouveaux groupes de *P. simus* du Nord par l'intermédiaire des communautés ont été discuté et admis comme une bonne stratégie ; toutefois, nous avons besoin plus d'une approche pour la sauvegarde de ces animaux gravement menacés (Randriarimanana, et al, 2012, Felaniaina, et al, 2012, Anjara et al., 2012). Nous avons beaucoup plus de connaissance sur *Prolemur simus*: cette espèce gravement menacée ne se reproduit pas bien en captivité (Rouillet, 2012) et la translocation de jeunes individus à partir des populations menacées pourrait être une meilleure approche pour la conservation de cette espèce. .

INTODUCTION

Le problème de conservation évoqué dans cette proposition concerne une espèce en danger critique, *Prolemur simus,* qui a été trouvé depuis longtemps à Talatakely dans le Parc National de Ranomafana. *Prolemur simus* est une espèce phare du Parc et attire beaucoup de touristes et des chercheurs. L'existence de cette espèce était parmi les critères de choix du Parc en site de Patrimoine Mondiale en 2007. Par ailleurs, une diminution en nombre de groupes et d'individus de *Prolemur simus* a été observée dans la forêt de Talatakely en 2006 (Wright et. al., 2008).
Wright et. al. (2008) a attiré l'attention des chercheurs et des Organisations-Non-Gouvernementales œuvrant dans le domaine de l'environnement et de la conservation qu'il y avait seulement 100 individus connus de *P. simus* dans le monde. A partir de ces informations, des recherches sur toutes les populations restantes de *P. simus* ont commencée dans des régions susceptibles de les trouver. Des populations de *P. simus* ont été trouvées à Ivato près de Farafangana, aussi bien qu'à Torotorofotsy et autour d'Ambatovy proche d'Andasibe. En 2008, à peu près 1 km en dehors de la limite du Parc National de Ranomafana, des groupes de *P. simus* ont été localisés à Vohitrarivo (groupes de 45 et 48 individus), et à Sahofika (autour de 35 individus).
En 2008 l'équipe de recherche du Centre ValBio ont pu observer trois nouveaux groupes de Prolemur simus dans la forêt fragmentée d'IVATO, près de Karianga.
Quatre individus ont été aperçus brièvement à Antanifotsy en 2010 et ce groupe a été suivi pendant le projet de conservation HELP Simus

.

En 2010 L'équipe de Biodiversité du Centre ValBio, en faisant un suivi dans la forêt d'Ivato ont découvert trois groupes différents (respectivement 31, 12 , 26 individus). Nous avons travaillé an étroite collaboration avec le village d'Ivato en créant des pépinières et avons engagé des habitants de la région en 2009-2010 pour suivre les animaux. Cette étude s'est poursuivie jusqu'en 2015. Grâce à la collaboration du CVB avec le village d'Ivato, P. simus n'ont pas été blessés et le nombre de la population a augmenté et est devenu 46, 16 et 50. Les habitants d'Ivato se sont plaints du fait que les P. simus mangent leur plantation (cannes à sucre, cafés, riz et manioc) et ils nous ont demandé d'emmener quelques individus de P. simus.

Rappel sur Prolemur simus

Hapalemur est réputé surtout par son nom lémur bambou à cause de son régime alimentaire qui est constitué principalement de bambous. Ce genre de lémurien se diversifie en 7 espèces et sous espèces avec des caractères adaptatifs différents dans leur aire de distribution à savoir *Hapalemur meridionalis, Hapalemur aureus, Hapalemur griseus occidentalis, Hapalemur griseus ranomafanensis, Hapalemur griseus griseus et Hapalemur alaotrensis, Hapalemur simus ou Prolemur simus* (Mittermeier et al, 2011). Comme d'autres lémuriens, ces espèces sont menacées d'extinction à cause de la dégradation dramatique de leur habitat naturel, suite aux activités excessives et irrationnelles des humains. Ces derniers chassent directement les lémuriens pour les vendre ou les manger ; transforment les grands arbres en bois de construction, d'ébénisterie et pratiquent la culture vivrière sur brulis (tavy). Par conséquent des forêts deviennent ouvertes, se fragmentent, diminuent de surface, perdent un grand nombre de plantes nourricières des animaux comme les bambous et la forêt est remplacée par des « savoka » ou terrain de culture.

Pour *Prolemur simus* la situation est alarmante car elle n'est plus présente que dans les 1-4% de son aire de distribution historique et l'étude montre que c'est une espèce spécialiste de nourriture et d'habitat c'est-à-dire qu'elle mange principalement d'une espèce de bambou appelée *Cathariostachys madagascariensis* et elle est aussi sensible à l'effet de lisière et adore le cœur de la forêt (Petter et al, 1977). D'où la taille de sa population devient très petite, environ 12 groupes, soient 100 individus sont enregistrés dans leur aire de distribution actuelle.

A propos du statut de conservation, *Prolemur simus* est gravement menacée (Wright et al, 2008). Ainsi la gestion en captivité et la gestion de population dans la nature sont recommandées pour sauver le reste de la population {CAMP (2001) ; Mittermeier et al, (2011) ; IUCN (2012}.

Pour le projet de conservation ex situ de Prolemur simus, 39 individus seulement sont élevés en captivité dans 22 institutions à Madagascar et en Europe à savoir Parc d'Ivoloina, Parc de Paris, Parc de Colonne...etc. Quant au projet de conservation in situ, des efforts pour l'inventaire systématique des sites ont été effectués, l'étude génétique et d'autres recherches tant dans son habitat historique que dans d'autres sites. D'après les études antérieures, l'aire de distribution de *Prolemur simus* était la partie Nord, Ouest, Nord-Ouest, la portion Centrale et Est de Madagascar (Mittermeier et al. 2008). Actuellement cette espèce ne se rencontre que dans la forêt humide du Sud Est et du Centre Est de Madagascar. Au Sud elle est présente dans le Parc National de Ranomafana, le Parc National d'Andringitra, et cinq autres forêts non protégées entre autres Kianjavato, Karianga, Morafeno, Evendra, Mahasoa (Wright et al, 2008). Au centre elle se rencontre dans la forêt de Torotorofotsy près du site minier d'Ambatovy (Dodge 2005, King et all 2013).

Désormais, dans le Parc National de Ranomafana, la petite population de *Prolemur simus*, environ 20 individus de 3 groupes se dispersent souvent et dans le groupe de Talatakely qui est le seul groupe habitué des touristes et des chercheurs, on ne rencontre plus qu'un mâle adulte et sa fille, une jeune femelle. Le rétablissement de cette population est alors le but de ce projet de réintroduction des individus ou groupes de *Prolemur simus* des autres forêts environnantes dans le Parc National de Ranomafana pour former des groupes stables et productifs assurant la viabilité de la population

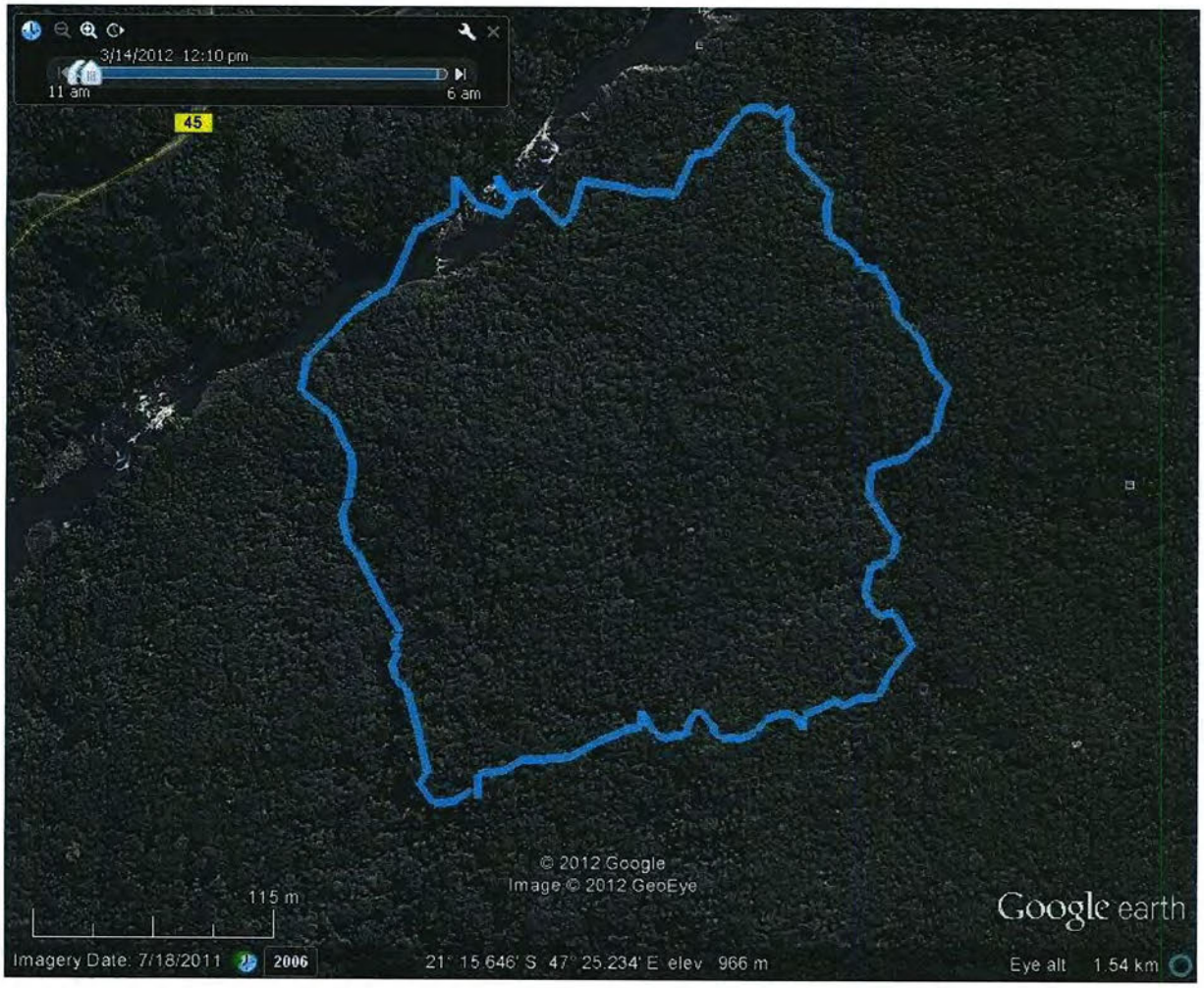
Les principaux objectifs sont d'améliorer la diversité génétique pour éviter la consanguinité et l'extinction de la population et maintenir l'équilibre de l'écosystème. En effet, le projet consisterait aux captures de *Prolemur simus* de la forêt d'Ivato, et réintroduction dans le site de Talatakely du « Parc National de Ranomafana ».

Hypothèse de disparition des *Prolemur simus* à Talatakely

Les raisons de la diminution en nombre de groupe et d'individus et de la disparition de ces individus de *P. simus* dans la forêt de Talatakely ne sont pas encore confirmées. Nous considérons quatre hypothèses possibles à vérifier ou confirmer dont la dégradation de l'habitat y compris la nutrition, la prédation, la génétique (consanguinité), l'effet de l'écotourisme (stress).

- *L'habitat (dégradation et nutrition)* :

95% de l'alimentation de *Prolemur simus* sont principalement constitués par le bambou *Cephalostachyum madagascarensis* (bambou géant) dans le Parc National Ranomafana. *Prolemur* mangent les feuilles mûres, les jeunes feuilles, la tige, la moelle et les jeunes pousses de *C. madagascariensis* (Tan, 1999, 2000). Les jeunes pousses contiennent une grande quantité de protéine et de la cyanure (Glander et al., 1992, Balhorn et al., 2010). Le comptage de tiges de bambou dans des plots botaniques effectué en 1997 à Talatakely avait montré une abondance relative qui varie de 0-36.6% de *C. madagascariensis* (Tan, 1999). En 2012 nous avons réévalué le nombre de tiges de *C. madagascariensis* à Talatakely et avons trouvé que l'abondance relative variait de 5-40%. Ces données montrent que l'abondance de bambou à Talatakely n'a pas diminué durant les 14 années passées et qui pourrait signifier que **l'abondance en bambou ne pourrait pas être la raison de la diminution en nombre de groupes et d'individus de *P. simus* dans le parc.**



Une des zones occupée (10 ha) par les bambous géants dans la forêt de Talatakely où le Prolemur simus fréquente.

Une autre zone occupée (9,8 ha) par des bambous dans la forêt de Talatakely où le Prolemur fréquente.

-La Prédation

Le prédateur principal des lémuriens est le *Cryptoprocta ferox* ou le Fosa. Pendant 26 années d'études de lémuriens à Ranomafana, le Fosa est reconnu comme prédateur des *Eulemur rubriventer*, des *Propithecus diadema* et des *Varecia variegata.* Nous n'avons pas eu l'évidence de prédation de *Prolemur simus* par le Fosa. A partir des photos prises par les « camera traps » il a été estimé qu'il y a 25 *Cryptoprocta ferox* dans le Parc National de Ranomafana (41,600ha) (Gerber et al., 2012). Dans d'autres sites, le Fosa mange des Sifakas des petits fragments de forêt (Irwin et al., 2010) ; pendant les 26 années d'étude continue sur *Propithecus edwardsi* dans le Parc National de Ranomafana, il y a eu 14 cas de prédation connus par le Fosa (Wright, 1998, Irwin et al., 2010). Il a été supposé que due à la nutrition de bambou qui contient une grande quantité de cyanure (une toxine qui tue des êtres humains) que consomme le *Prolemur simus*, ces *P. simus* ne pourraient pas être le choix de proie préférée par un prédateur. Depuis que le groupe de *P. simus* à Talatakely a été suivi au moins cinq jours par semaine durant les dix-sept dernières années, si *Cryptoprocta ferox* avaient tué des *P simus.*, les chercheurs auraient trouvé les cadavres, comme on trouve dans les cas des autres espèces de lémuriens. Donc, **on peut dire que la prédation est à exclure comme raison de la diminution en nombre de groupe et d'individus de *P. simus* à Talatakely.**

-La génétique (consanguinité)

Ce groupe de *P. simus* comprenait une mère, une fille et petite-fille comme femelles reproductrices. La progéniture mâle a quitté le groupe à l'âge de 3-5 ans pour avoir des occasions de reproduction dans les autres

groupes. Quand le mâle reproducteur qui joignait le groupe en 1999 a disparu en 2005, les femelles et leurs progénitures ont émigré pour trouver un autre mâle. Puis, les trois femelles reproductrices ont été disparues de Talatakely peut-être à la recherche de partenaires reproducteurs. Actuellement, les seuls individus de ce groupe de *P. simus* sont le père et sa fille. Dans ce cas, la reproduction serait quasiment impossible, et même s'il y aurait de reproduction, le risque de consanguinité serait très élevé. Par conséquent, la probabilité d'émigration de ces deux individus restant est très élevée aussi. **Le manque de diversité génétique pourrait être, alors, une des raisons de disparition d'individus de *P. simus* d'où la diminution en nombre de groupe et d'individus a Talatakely**. Ce serait, alors, le moment de prendre une décision de sauvegarde en faisant une translocation de quelques individus venant d'autres sites afin de chercher une nouvelle diversité génétique pour le groupe de *P. simus* dans la zone protégée de Talatakely.

-Les effets du tourisme (stress et présence humaine en abondance fréquente)

Nous avons effectué une étude de courte durée en 2011 en demandant les touristes les raisons principales de leurs visites au Parc National de Ranomafana. Cette étude montre que les lémuriens, en particulier, l' *Hapalemur* aureus, le *Propithecus edwardsi* et le *Prolemur simus,* sont la raison principale de leur visite au PNR. Beaucoup de touristes veulent voir le *Prolemur simus,* qui est une espèce en danger critique. 84% des touristes (n=245) ont vu *Prolemur simus* en 2011 en exprimant leur satisfaction de la visite à Ranomafana. Cependant, cette présence humaine en abondance fréquente pourrait créer des perturbations tant au stress des animaux qu'au piétinement des futures jeunes pousses de bambou. Est-ce-que ce serait, alors, une des raisons de disparition de groupe ou d'individus de *P. simus* ? Si oui, il faudrait, alors, effectuer une étude de comparaison des groupes de *P. simus* dans des zones plus fréquentées par l'être humain par rapport à celles qui sont moins fréquentées par les gens en utilisant des indicateurs de stress à travers les comportements des animaux ou les échantillonnages des indicateurs de stress comme la variation de taux d'hormones dans leurs corps. **L'évidence de l'effet du tourisme à la disparition du *Prolemur simus* de Talatakely est plus complexe voir moins convaincant, mais mérite des études plus approfondies à moyen ou long-terme afin de tirer des conclusions plus rationnelles.**

D'après les quatre hypothèses proposées ci-dessus, le manque de diversité génétique et le risque de consanguinité parmi les individus des groupes pourraient être plus convaincants quand à la raison de diminution en nombre de groupe et d'individus de Prolemur simus à Talatakely. Par conséquent, nous proposons fortement au Gestionnaire du Parc National de Ranomafana la translocation de quelques individus de P. simus venant du plus proche site et qui habitent dans un habitat un peu similaire à ceux de Talatakely afin de les réintroduire dans la forêt de Talatakely et de sauvegarder les deux individus y restant pour la continuité de la vie de cette population avec une diversité génétique meilleure.

Rappel sur la translocation et leçons apprises

En 2014, l'équipe de CVB a transféré trois Prolemur simus dans le Parc National de Ranomafana. Toutefois, ce transfert n'a pas réussi car aucun des trois individus n'est resté dans la zone. En effet, nous n'avions pas pu les suivre car leurs colliers émetteurs ont relâchés.

Les potentielles raisons de cet échec ainsi que les recommandations pour réussir ce projet seront énumérées ci-après.

Les critères de réussite sont la création d'un nouveau groupe de Prolemur simus au sein du groupe. Parc national de Ranomafana et, éventuellement, la dispersion de certains individus dans le groupe existant (père et fille) de Prolemur simus du Parc National de Ranomafana

Erreur: Nous avons capturé trois individus pour la translocation
Recommandations: Les experts conseillent qu'au moins 6 à 16 individus soient transférées pour réussir.
Projet de translocation 2019: Nous allons transférer au moins 6 individus, mais nous espérons que 16 individus du site d'Ivato seront transférés dans le Parc National de Ranomafana .

Erreur: nous avons capturé trois individus appartenant à trois groupes différents de P. simus

Recommandations: Tous les individus capturés doivent appartenir au même groupe.

Projet translocation 2019: nous allons transférer ensemble tous les individus du même groupe

Erreur: Nous n'avons pas observé les individus avant la translocation.

Recommandations: Il faut effectuer le suivi des individus afin d' observer les relations et la santé des individus pendant 10 jours à deux semaines.

Projet translocation 2019: Nous allons suivre les individus pendant dix jours à deux semaines avant la capture pour choisir les meilleurs individus à transférer.

Erreur: Nous avons laissé les 3 individus dans la cage d'habituation pendant la nuit dans le RNP, puis les avons relâchés le lendemain.

Recommandations: Il faut laisser les individus s'habituer à l'intérieur de la cage pendant environ 2 à 4 semaines afin de les laisser s'adapter à l'environnement.

Projet translocation 2019: Nous allons garder les individus transférés dans la cage pendant au moins deux semaines, en les nourrissant avec du bambou et d'autres aliments trois fois par jour.

Erreur: la taille des colliers utilisés était trop grande et ils sont relâchés pendant les déplacements des individus

Recommandations: La taille des colliers utilisés devrait être appropriée pour chaque individu

Projet de translocation 2019 : Nous placerons des colliers appropriés sur chaque individu pour le suivre après la relâche.

Nous assurerons que chaque animal se nourrit correctement. Nous surveillerons également la charge parasitaire et la santé des animaux pendant ces deux semaines. Nous allons séparer les animaux qui tombent malades ou qui ne mangent pas bien dans une cage plus petite et les surveiller de plus près.

Relâche: une fois les individus transférés relâchés, ils seront suivis pendant dix jours consécutifs avec des équipes tournantes d'observateurs du CVB. Six-quatorze techniciens seront employés pour suivre chaque individu transféré de l'aube au crépuscule, en prenant des données sur le comportement, l'alimentation, les interactions avec d'autres individus.

Suivi: après la période de dix jours, ces animaux seront observés cinq jours par semaine. Un rapport hebdomadaire sera remis à MNP et CVB. Tout comportement de reproduction et/ou d'accouplement observés seront notés.

Importance de la translocation

Bien que nous avions suivi les populations de lémuriens à Ivato de manière intermittente depuis 2008, les communautés locales nous ont récemment demandé d'enlever certains animaux afin de reduire le nombre d'animaux dans la zone parce qu'ils détruisent leurs cultures. Jusqu'ici, nous avons maintenu de bonnes relations avec les villageois et ils se sont abstenus de tuer les lémuriens, mais nous craignons qu' ils vont perdre patience si on ne prend pas une décision. Cette situation est une des raisons pour effectuer la translocation dès que possible.

Une autre raison d'effectuer la translocation le plutôt possible mais de ne pas attendre novembre est la saison de naissance de Prolemur simus. Il est dangereux de capturer et d'anesthésier les femelles en fin de leur grossesse. De ce fait, il est important d'effectuer la translocation le plus tôt possible afin de pouvoir capturer les femelles avant la période de reproduction.

L'une des principales préoccupations concernant la translocation de Prolemur simus est le « homing » c'est-à-dire le comportement de ces lémuriens à vouloir se retourner dans leur zone natale. Cependant, il est important de noter que sur la base des expériences et des discussions avec des experts en translocation, le principal facteur déterminant chez les animaux effectuant un tel retour semble être la disponibilité des ressources, et non les changements d'altitude ou de température. De ce fait, la saisonnalité de la translocation de Prolemur simus ne devrait pas être une préoccupation car un soin particulier a été pris pour s'assurer que le nouveau groupe sera satisfait des ressources disponibles dans le Parc National de Ranomafana.

Les lémuriens, comme tous les autres animaux, peuvent s'adapter aux changements de température dans la mesure où ils sont maintenus dans l'aire de répartition naturelle de l'espèce. En effet, comme l'altitude de repartition de P. simus a été déjà connue (entre 28 m et 1 600 m selon Ravaloharimanitra 2011, Wright 2008), l'altitude de Talatakely, 900m, est comprise dans cette marge. De plus, Talatakely abrite un groupe de P. simus depuis longtemps. Les experts du Centre ValBio ont observé directement, pendant 32 ans, que P. simus réussissait bien dans le Parc, quel que soit la saison et les fluctuations de température (Wright 2008). Puisque P. simus réside dans Parc National de Ranomafana, nous savons que l'espèce possède les adaptations nécessaires, fourrure épais et longue queue, pour s'adapter aux températures de Talatakely. Ces lémuriens régulent leur température grâce à des comportements tels que prendre un bain de soleil, s'entasser et par l'érection des poils de la queue, qui fournit une couche isolante d'air à proximité de la peau. La queue peut ensuite être utilisée comme une écharpe et enroulée autour du lémur pour l'isolation du reste du corps. Comme aucun de ces mécanismes de thermorégulation n'est saisonnier, les lémuriens sont capables de s'adapter rapidement aux changements de température (Chaplin 2014).

Nous prévoyons de capturer le groupe de lémuriens d'Ivato / Karianga à une altitude d'environ 300 m et de les transporter à une altitude d'environ 900 m de Ranomafana. Cette différence de changement d'altitude de 600 m est faible si l'on considère que l'habitat naturel de P. simus s'étend sur près de 1 600 m. Ce changement d'altitude ne provoque qu'un léger changement de température : en moyenne, Ivato a une température d'environ 17 ° C tandis que Ranomafana est d'environ 15 ° C . Même si nous ne sommes pas préoccupés par le changement de température des lémuriens, ces animaux bénéficieront d'une période d'acclimatation de deux à quatre semaines pour s'adapter au nouvel environnement, température comprise, avant d'être libérés.

En nous concentrant sur le principal facteur motivant le retour des animaux transférés dans leur domaine d'origine, nous avons pris des mesures pour nous assurer que les lémuriens seront satisfaits de la nourriture disponible à Ranomafana et ne seront donc pas enclins à quitter la zone. Premièrement, nous avons mené une enquête sur *Cathariostachys madagascariensis,* le bambou de choix de P. simus, dans la région de Talatakely où nous prévoyons que le nouveau groupe de lémuriens choisira son nouveau domaine vital. Le transect a été réalisé sur des parcelles de 10 m sur 10 m sur une portée de 20 m sur 300 m et comprend l'emplacement de la cage d'acclimatation. Il est important de noter que les cours d'eau ont croisé le transect plusieurs fois et qu'il existait des parcelles de bambou extrêmement denses adjacentes aux transects puisqu' on sait que le bambou se développe le long des cours d'eau. Nous avons déterminé que Talatakely contient suffisamment de *Cathariostachys madagascariensis* pour accueillir 16 autres P. simus. De plus, une surabondance de cette espèce de bambou a été observée dans les forêts de Ranomafana en dehors des spécifications du transect et, par conséquent, les lémuriens auront une nourriture abondante même s'ils choisissent une zone différente pour s'habituer.

D'après les observations des populations de P. simus à Ivato, nous savons que ces individus sont adaptés pour manger non seulement les espèces de bambou typiques, mais aussi pour le riz, les tiges de manioc, le café et les tiges de gingembre des agriculteurs locaux. Ces aliments ne font pas partie du régime naturel de P. simus et le bambou du Parc National de Ranomafana est meilleur pour la santé des lémuriens. Au début de la période d'acclimatation, nous proposerons aux lémuriens un menu de choix en plus de leur régime alimentaire naturel à base de bambou. Nous espérons qu'ils choisiront simplement de ne pas consommer les suppléments si leur alimentation naturelle leur est offerte en abondance. Toutefois, s'ils manifestent le désir des cultures, nous continuerons d'ajouter des cultures à leur régime alimentaire, puis de les sevrer lentement des cultures pendant

la période d'acclimatation, jusqu'à ce qu'ils mangent à 100% de nourriture naturelle. En effectuant ceci, les lémuriens pourront s'ajuster lentement à leur alimentation naturelle plutôt que de subir un changement brutal, ce qui signifie qu'ils seront beaucoup moins susceptibles de quitter Ranomafana à la recherche de suppléments de culture.

Une dernière considération pour la période de transfert est la sécurité de l'équipe chargée de l'exécution du projet. Etant donné que les routes menant à Ivato est dans un si mauvais état, la pluie au mois de novembre rendrait les routes encore plus dangereuses à traverser. Il ne serait pas bien de mettre les personnes et les lémuriens à un risque inutile lorsque le projet peut être réalisé à un moment plus sec et donc plus sûr. Il ne serait pas conseillé de transférer les lémuriens en novembre en cas de retard dû au mauvais état des routes. Le temps supplémentaire de déplacement serait dangereux pour leur santé et ne serait pas dans leur meilleur intérêt.

La translocation de P. simus dans le Parc National de Ranomafana est importante pour la conservation de l'espèce et nous voulons donc nous assurer que tout est planifié soigneusement, ce qui inclut le moment choisi. Tenant compte des moyens de subsistance menacés par la population locale, de la disponibilité des ressources, des mécanismes de thermorégulation des lémuriens et de la sécurité des personnes et des lémuriens concernés, nous estimons que le transfert devrait avoir lieu dès que possible et ne devrait pas attendre novembre.

METHODOLOGIE

1. Sites d'études

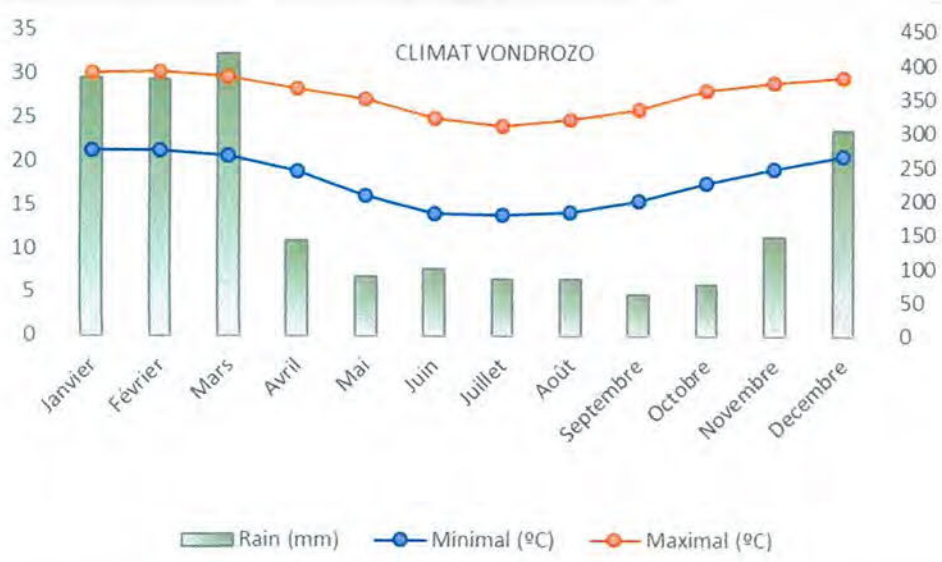
a. Site de capture « Forêt d'Ivato » Le choix de site de capture pour la translocation est important car ce site doit être inclus dans l'aire de distribution de l'espèce cible selon la recommandation des spécialistes de réintroduction de l'IUCN. Il vaut mieux de choisir un site le plus proche du site de translocation pour éviter le risque de stress de l'animal après la capture, le déplacement jusqu'à l'heure de la relâche.

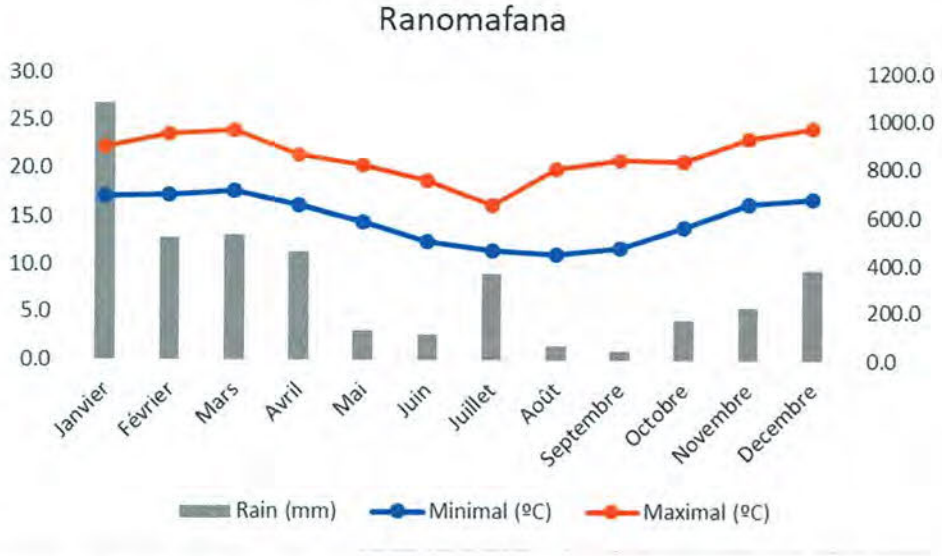
Site de collecte: Forêt d'Ivato:

MAHASOA:	Lat: -22.422117°	Long: 47.283968°
MANGARABAKA:	Lat -22.419310°	Long: 47.281110°

Ce site est degradé et fragmenté avec trois petites forêts de 5ha, 7ha and 10ha. Les bambous sont présents mais à cause de la réduction de la surface de la forêt, Prolemur simus se déplacent dans les champs et mangent les maniocs, les canne à sucre et le riz.

Climat à Ivato (précipitations et température) par rapport à celui de Ranomafana

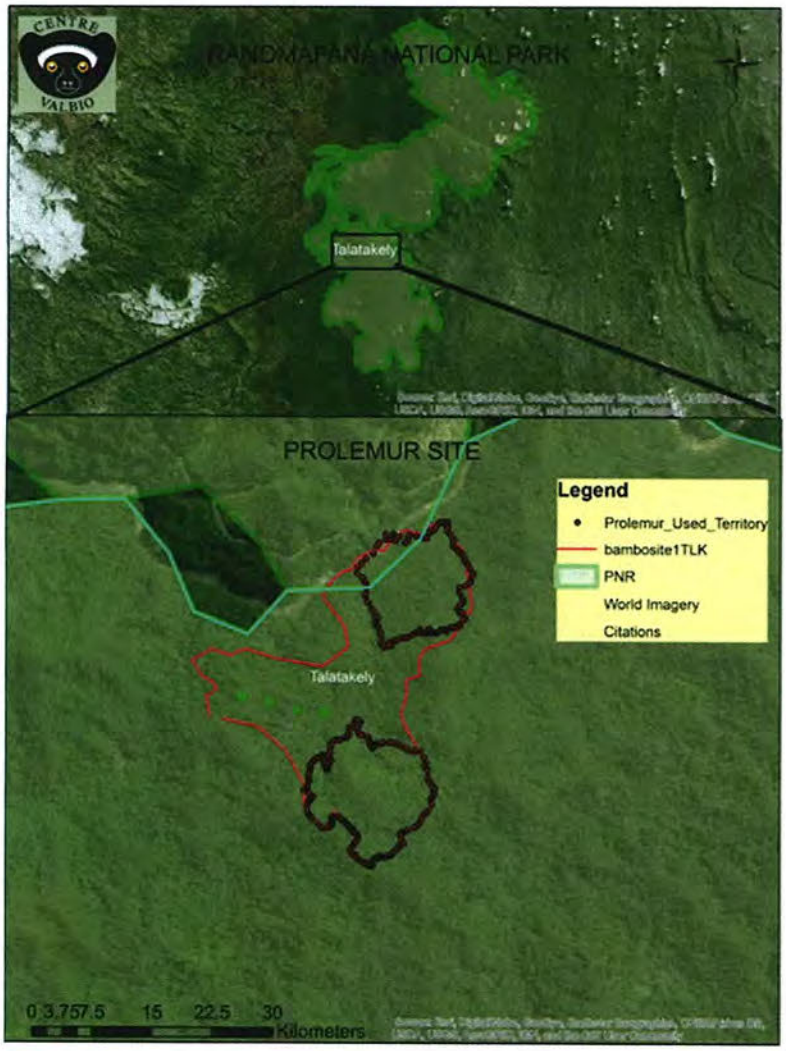


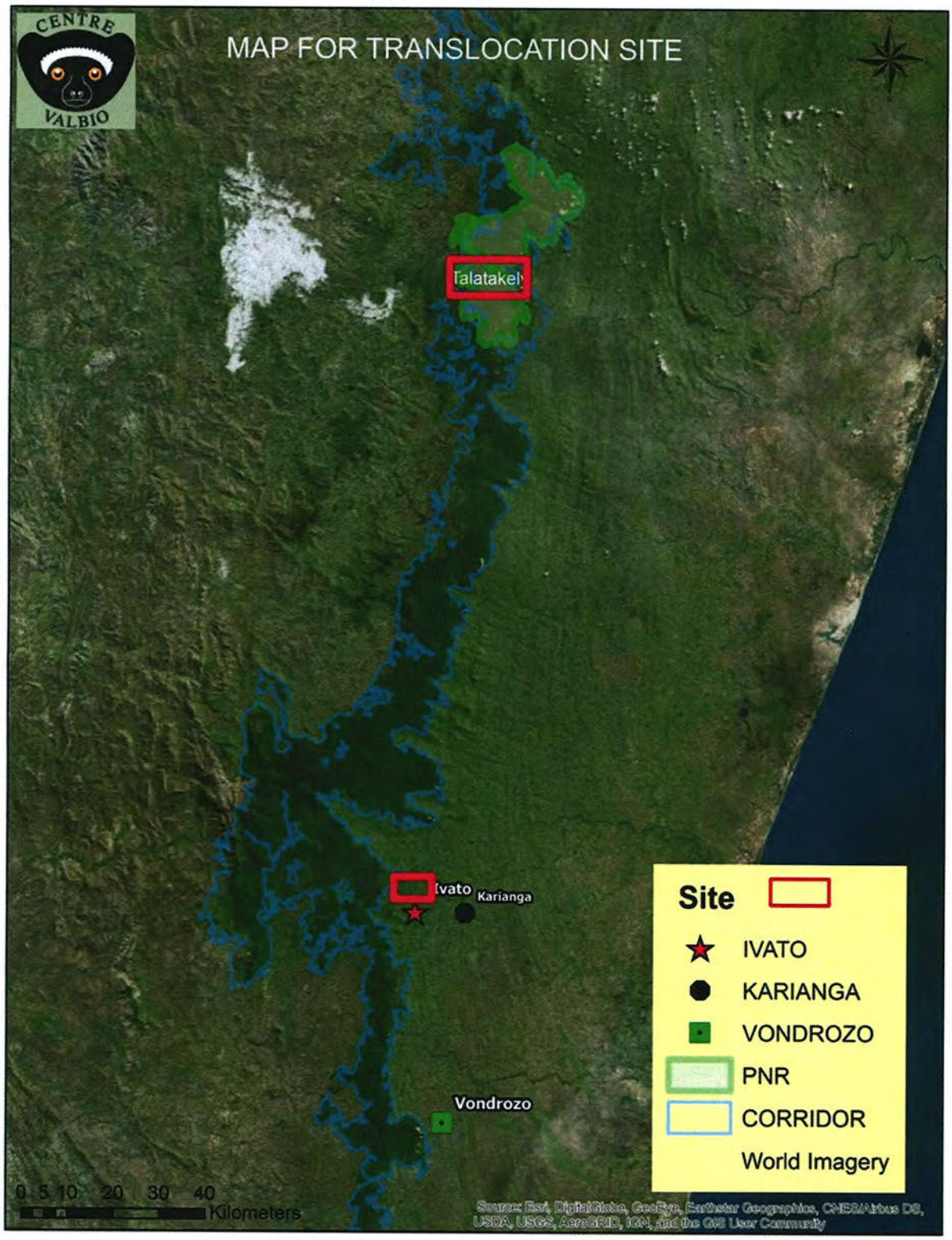


Bien que Ranomafana reçoive des précipitations plus importantes (ce qui se traduit par une croissance plus importante du bambou), Ranomafana et Vondrozo ont des températures très similaires. En moyenne, Ranomafana n'a que quelques degrés de moins que Vondrozo.

b. Site de translocation/ relâche « FORET DU PARC NATIONAL RANOMAFANA »La forêt de Ranomafana est l'habitat historique de Prolemur simus. Elle est une aire protégée depuis 1991 après la découverte de la nouvelle espèce de lémuriens appelée « Hapalemur aureus » par Dr Patricia Wright, promoteur du Projet Parc National de Ranomafana. Elle est parmi les aires protégées les plus visitées à Madagascar ce qui répond à la revendication de groupe de spécialiste de l'IUCN. D'après eux, un projet de réintroduction est possible pour un site à la fois habitat historique de l'animal et protégé pour la conservation de la nature. Cette forêt est particulière par la gestion durable de Madagascar National Parks et aussi le partenariat avec le centre ValBio et l'ICTE/MICET qui appuient les projets de recherche, éducation et développement économique et social de la population locale. Le site de relâche devrait être typique du microhabitat de Prolemur simus pour répondre aux besoins biologiques et écologiques de cet animal. Ce site serait donc riche en bambou « Cathariostachys madagascariensis »

composant essentiels de son régime alimentaire. Les espèces nourricières suivantes sont aussi compléments de son alimentation en milieu naturel : fleur de Ravenala madagascariensis, fruit d'Artocarpus integrifolia, Ficus spp. et Dypsis spp., et feuille de Pennisetum clandestinum (Meier and Rumpler, 1987). Le site n'est pas proche de la limite de la forêt pour éviter la fuite ou la sortie de l'animal ou le retour de l'animal au site de capture. De plus, le site de relâche ne devrait pas chevaucher avec le territoire du groupe résidant, surtout dans domaine vital du groupe existant pour éviter le combat entre les individus résidants et le nouveau groupe. Il faut rappeler que le domaine vital est l'endroit le plus fréquenté c'est à dire l'endroit où il dort, se nourrit, se défend contre le prédateur, se reproduit etc. Pour l'adulte male à insérer il vaut mieux de le relâcher sous les yeux de groupe existant (l'adulte mâle et la jeune fille) afin d'éviter la désorientation de ce mâle solitaire. Ce dernier aura besoin de l'aide du groupe existant pour se défendre contre le prédateur, pour faciliter la quête de nourriture. En outre, étant dominant de son group d'origine, il est fortement probable que le nouvel adulte mal puisse s'insérer dans le groupe existant et s'y domine pour se reproduire avec la jeune fille.




CENTRE VALBIO
MAP FOR TRANSLOCATION SITE
Talatakely
Ivato
Karianga
Vondrozo
Site
IVATO
KARIANGA
VONDROZO
PNR
CORRIDOR
World Imagery
0 5 10 20 30 40
Kilometers
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

2. Espèce étudiée, choix des individus capturés
    a. Espèces étudiées- D'après les spécialistes de réintroduction de l'IUCN, la faisabilité de réintroduction exige la connaissance de l'espèce en question c'est pourquoi cette partie parle surtout de la biologie et écologie de Prolemur simus. Selon le nom vernaculaire, cette espèce est la plus grande des espèces mangeurs de bambous. En moyenne, son poids atteint 2.2 à 2.8kg (Mittermeier et al. 2006) et 2.365kg d'après Glander et al. (1989). La longueur du corps peut atteindre 40 à 42cm avec une queue de longueur assez élevée de 45 à 48cm (Mittermeier et al. 2006). Le corps est de couleur brun vert avec le dessus de la tête et le dos gris brun, la queue est de même couleur que le corps avec une extrémité brun noire; la tête, le cou, les épaules et les bras supérieurs en brun olive. Sa face est large et émoussée avec un museau court et large (Mittermeier et al. 2006). Il n'y a aucun dimorphisme sexuel concernant le poids corporel et le sexe (Tan, 1999). C'est une espèce cathémerale et vit en groupe de 2 à 12 individus jusqu'à 30 individus (Rowe,1996). Elle est constituée de multifemelles adultes et ses jeunes et un ou deux mâle adulte reproducteur. Elle dure 149 jours (Tan, 1999). La période de mise bas est à partir du mois de novembre. (Tan, 2000). Dans le Parc National de Ranomafana *Prolemur simus* vit en cohabitation avec Hapalemur griseus et Hapalemur aureus. Leurs régimes sont basés sur des bambous appartenant à la famille de Poaceae. Parmi les six espèces de bambous qui y existe, les quatre sont consommées par les espèces de l'Hapalemur entre autre : Cathariostachys madagascariensis appelé Volohosy, Cephalostachyum perrieri dite Tsimbolovolo lavalava ravina, Cephalostachyum viguieri nommé Tsimbolovolo boribory ravina; Poecilostachys festucaceus dite Vilon'ala. Les parties consommées de chaque espèce varient suivant le mois et la saison. En plus du bambou, ils mangent des fruits, des feuilles et des pétioles d'autres plantes. Il faut noter que la cohabitation des espèces dans un habitat est réglée par la séparation des niches écologiques ; c'est-à-dire que si elles ont le même régime alimentaire, rythme d'activité, niveau fréquenté, la partie consommée peut varier entre elles.
    b. Individus capturés pour la translocation- Les individus capturés seront les compléments du groupe résident (adulte mâle et jeune fille).Comme nous savons que Prolemur simus vit en un large groupe, au moins 2 mâles et 4 femelles seraient donc nécessaires pour la viabilité de ce groupe, mais en plus est meilleur.
    -Un des nouveaux mâles devrait être dominant dans son groupe d'origine et ou être de grande taille pour faciliter l'accueil par la femelle résident.
    -Chez Prolemur simus, donc il devrait y avoir aussi au moins six autres femelles dans le groupe
    -Une paire male-femelle dominant de son groupe d'origine serait également proposée pour diminuer le stress et éviter la désorientation et la prédation.
    -Un total d'au moins 15 individus seront donc nécessaire, toutefois trois mâles et 12 femelles seraient l'idéal pour la réussite ce projet.

3. Procédures : Les procédures de réintroduction nécessitent non seulement une étude préalable des animaux à capturer mais également de l'endroit où les animaux seront introduits.

Etudes avant la réintroduction

Rappel concernant Prolemur simus à Ivato (2008 -2019)

En 1997, pendant l'expédition de Steig Johnson, l'équipe du CVB et Pat Wright ont aperçu Prolemur simus d'abord à Ivato, une région située juste au nord de Vondrozo et à un kilomètre du corridor CoFav. Cette région possède des fragments de forêt de 2 à 10 ha. En 2008, une équipe du CVB dirigée par Eileen Larney et Rachel Jacobs est revenue à Ivato et a observé un groupe de plus de 30 individus. En 2009 et 2010, l'équipe du CVB, dont Patricia Wright, a créé des pépinières et enseigné les techniques de reboisement, ainsi que des techniques de collecte de données de l'écologie comportementale de Prolemur simus aux membres de la communauté locale. Les guides ont continué à prendre des notes sur l'alimentation et le comportement des nouveau-nés de 2008 à 2010. L'équipe de CVB a effectué des voyages annuels à Ivato pour former la population locale sur la collecte de données. En 2014, le nombre d'individus de Prolemur simus était passé à 71. En 2016, nous continuions encore à enseigner à la communauté locale la collecte de données Prolemur simus, mais nous avons constaté des problèmes de tavy et de piégeage des lémuriens. Une grande partie du territoire des lémuriens a été détruite et remplacée par des

cultures. En mars et avril 2018, 5 fokontany ont convoqué une grande réunion pour se rendre compte du problème des lémuriens en danger et ont décidé de donner à CVB la permission de gérer le site et les lémuriens. En décembre 2018, la communauté d'Ivato a écrit une lettre officielle à CVB concernant leur collaboration avec CVB pour la conservation des lémuriens. , la population de Prolemur simus dépasse les cent individus et les lémuriens mangent parfois du manioc, du riz, jacquiers, du café, de la canne à sucre et d'autres plantes. En transférant un groupe de 15 lémuriens, nous soulagerons une partie de la pression exercée par ces lémuriens dans cette région, de sorte que les individus restant puissent être en meilleure santé.

Régimes alimentaires de Prolemur simus : études botaniques dans le Parc National de Ranomafana:

Pour répondre aux besoins nutritionnels des Prolemus simus d'Ivato, nous avons fait appel à une experte en nutrition, Amanda DuBour, et avons pris des mesures pour que les animaux transférés aient suffisamment de nourritures à Talatakely, dans le parc national de Ranomafana. Tout d'abord, nous avons mené une étude sur Cathariostachys madagascariensis, l'espèce de bambou préférée de P. simus, dans l'ensemble de la région de Talatakely, où nous prévoyons que le groupe de lémuriens transférés établira leur domaine vital. Selon Chia Tan 1999, le régime alimentaire normal de P. simus en bonne santé dans un habitat de forêt primaire est composé à 95% de C. madagascariensis. Le transect botanique a été réalisé sur une parcelle de 20 m sur 300 m à Talatakely et comprend l'emplacement de l'enceinte de P. simus. Les cours d'eau ont croisé le transect plusieurs fois et le bambou prospère le long des cours d'eau. Des parcelles denses de bambou existent également à proximité du transect. Nous avons déterminé que Talatakely contient suffisamment de C. madagascariensis pour accueillir 16 autres P. simus. De plus, une abondance de cette espèce de bambou a été observée dans les forêts de Ranomafana en dehors des limites du transect et, par conséquent, les lémuriens auront suffisamment de nourriture.
En raison de la culture sur brûlis entourant le fragment de forêt d'Ivato où vivent les lémuriens, ces individus sont non seulement adaptés à la consommation d'espèces de bambou, mais aussi à d'autres aliments comme le riz, les tiges de manioc, le café et les tiges de gingembre (longoza) des agriculteurs locaux, menaçant les moyens de subsistance des populations locales et les poussant à demander le retrait des lémuriens. Ces aliments ne font pas partie du régime naturel de P. simus et le bambou de la RNP est meilleur pour la santé des lémuriens. D'après les données collectées à Ivato sur une période de trois ans de 2008 à 2010, nous savons que le régime alimentaire de P. simus à Ivato est en majorité constitué de bambou. Au cours des trois années de collecte des données, environ 74% du régime des lémuriens était composé en moyenne de bambou volojatsy (Valiha diffusa). L'espèce de bambou préférée de P.simus (C. madagascariensis) n'est pas disponible dans les fragments de forêt d'Ivato. Cependant, le schéma d'un régime majoritairement bambou chez P. simus d'Ivato est un bon signe qu'ils vont bien s'adapter aux bambous de Ranomafana où il existe de nombreuses espèces de bambou disponibles pour les lémuriens. Les données collectées de 2008 à 2010 démontrent un degré élevé de flexibilité dans le régime alimentaire des lémuriens. En effet, au cours de ces trois années, on a observé que les lémuriens consommaient des plantes de huit espèces différentes. Bien que V. diffusa reste la plus grande partie du régime alimentaire au cours des trois années, la nourriture constituant le reste du régime varie considérablement. Les habitudes alimentaires mises en évidence par cette population démontrent un degré de flexibilité qui indique de manière positive que les nouveaux lémuriens s'adapteront bien au régime Ranomafana. Certaines de ces espèces supplémentaires, notamment les longoza (Aframomum angustifolium) et les voatrotrok'ala (Clidemia hirta), qui constituaient respectivement environ 37% et 13% de leur régime alimentaire en 2008, sont également abondamment trouvées à Ranomafana, offrant des options plus familières aux lémuriens après translocation.
Au début de la période d'acclimatation à l'intérieur de l'enceinte, nous proposerons aux lémuriens un menu de choix en plus de leur régime alimentaire naturel à base de bambou (longoza, concombre, voatrotrok'ala, etc.). Nous leur donnerons tout le temps dont ils ont besoin pour adapter leur régime alimentaire.

Pour bien comprendre la distribution de la nourriture de ces lémuriens, les agents de MNP et du Centre ValBio ont effectué des reconnaissances et ont choisi sites pour des transects botaniques. Les résultats de cette étude de distribution de bambou (Cathiostachyium vigueri) effectuée en 2019 montrent qu'une quantité suffisante de bambous existe encore dans le territoire du groupe dans le Parc.
Une étude de l'endroit pour mettre les P. simus dans le Parc a été effectué par les agents de MNP et Centre ValBio. Un enclos temporaire de 15m x 4m x 15m de haut fait par des grillages sera construit dans le parc pour

y habituer les P. simus capturés. Cet enclos serait établi dans un endroit plat entre les rivières de Sakaroa et de Mariavaratra, loin des touristes sur la piste P. Les avantages de cet endroit sont l'abondance des bambous et l'absence de gros arbres qui devraient être coupés pendant la construction de l'enclos. Cet endroit est facilement accessible à partir des principales pistes mais loin des endroits fréquentés par les touristes.

50 échantillons de matières fécales seront prélevés approximativement pour analyser les espèces de parasites existants et leur prévalence. Les individus seront suivis afin de décider qui sera choisi pour le transfert. Simultanément, l'équipe technique du CVB, suivrons le groupe *Prolemur simus* dans le Parc National de Ranomafana.

Capture des animaux à l'extérieur du Parc
Au moins 15 individus d'un groupe de la forêt fragmentée d' Ivato seront capturés, examinés par un vétérinaire pour savoir leur état de santé, marqués par un collier émetteur seront transférés à Ranomafana. Nous ferons attention de ne pas capturer les animaux enceintes. Nous savons que *Prolemur simus* n'est pas monogame et forme un grand groupe qui peut atteindre jusqu'à 11 – 38 individus avec un territoire jusqu'à 50 ha (Wright et al., 2008, Louis et al., 2012). Nous savons également que la reproduction ne se passe pas dans un petit groupe et la compétition entre mâles augmente la reproduction (Wright, et al, 2008, Roullet, 2012).

a. Capture et Examen Physique
Le détail de la méthode de capture et le protocole d'évaluation sont décrits par Junge et al (2008). Chaque individu sera capturé à l'aide de fusil à flechette utilisant du Telazol à raison de 10 mg/kg (Dan-Inject MJ model, Dan-Inject, Knoxville, TN) (Type C Disposable Dart, Pneu-Dart, Williamsport, PA). L'effet de produit dure 2à 5mn pour faire l'examen physique et le prélèvement des échantillons (tissus, sang) . Pour l'examen physique les paramètres suivants sont enregistrés: poids, température, pulsation, respiration, heure de capture (heure et minute), tissu prélevé (nombre et taille), sang prélevé, dose totale de l'anesthésie utilisée, Mensuration du membre antérieure (humérus, radius/ulna, main etc....) et du membre postérieur (Fémur, tibia/péroné), pieds etc...., référence de puce implanté, canine supérieure, canine inférieure

b. Prélèvement des tissus
Pour une analyse génétique, quatre biopsies de 2.0 millimètres seront prélevées à partir de chaque animal. Ces échantillons sont stockés dans des tubes à température ambiante (Longmire et al, 1992). En plus, 1.0 cc de sang par kilogramme de poids corporel sera également collecté pour une analyse biomédicale. Une puce électronique sera implantée en sous-cutanée entre les omoplates de l'animal pour l'identification permanente de chaque individu. Un collier émetteur de faible fréquence serait posé sur le cou de l'animal afin de le suivre après la relâche. La fréquence du collier de chaque animal est vérifiée sur place avec l'utilisation d'une antenne réceptrice.

Dr. Wright, une équipe de techniciens du Centre ValBio, les vétérinaires malgache et les étudiants Américains et Malagasy se lanceront dans une expédition dans le village reculé d'Ivato. L'équipe campera à Ivato pendant deux semaines, observera le comportement des lémuriens et collectera des échantillons de matières fécales pour déterminer la présence de parasites afin de sélectionner un groupe de 15 lémuriens qui aurait les meilleures chances de réussir à cette translocation. Nous apporterons des lames et un microscope pour Ezzeldin pour analyser les échantillons de matières fécales de tous les lémuriens potentiels pour le transfert afin qu'ils puissent être évalués et traités des parasites. Lorsque nous serons sûrs que les individus d'Ivato sont en bonne santé, nous les capturerons avec des fléchettes anesthésiques avec Telazol conformément au protocole d'anesthésie de lémuriens du Centre ValBio et l'équipe de capture utilisera des filets pour assurer que les lémuriens soit en sécurité pendant la capture. L'équipe de capture du CVB et les vétérinaires vérifieront leur état de santé. Bekah et le vétérinaire feront le suivi de l'anesthésie, les examens physiques complets (y compris les prise de températures, le rythme cardiaque et respiratoire), les prises de sang, la pose des collier. Le sang sera prélevé sur les lémuriens sous sédations et placé dans des tubes de sang héparinisés et des tubes de sang contenant de l'acide éthylène diamine tétra-acétique (EDTA). Le sang héparinisé sera mis dans une centrifugeuse et le plasma sera prélevé à la pipette puis transféré dans un cryotube qui sera placé dans de l'azote liquide pour stockage. Nous aurons également des tubes d'hématocrite, un plateau en argile de scellement, une carte de lecture en volume des cellules emballées (PCV), un réfractomètre et une centrifugeuse portable. Avec ces appareils, les responsables pourraient commencer les analyses des échantillons sur terrain. Toutefois, une partie des échantillons sera transportée aux laboratoires du Centre ValBio et /ou exportée aux USA pour des analyses supplémentaires. Les formulaires pour les données collectées seront présentés en pièces jointes.

c. Transfert à Ranomafana et acclimatation /habituation: Chaque animal est placé dans une cage en plastique où il recevra du bambou pendant toute la durée du voyage. L'équipe assurera le suivi des animaux pendant leur transfert à Ranomafana où ils seront placés temporairement dans un enclos pour permettre l'acclimatation et pour le suivi de leur santé. En effet, avec l'approbation des responsables de Madagascar National Parks, les animaux seront transférés dans un enclos à l'intérieur du Parc pour commencer l'habituation. L'équipe surveillera la santé des lémuriens pendant leur acclimatation/habituation afin de s'assurer qu'ils mangent bien et se comportent bien. Ils seront nourris quotidiennement dans l'enclos jusqu'à leur libération dans le parc national de Ranomafana.

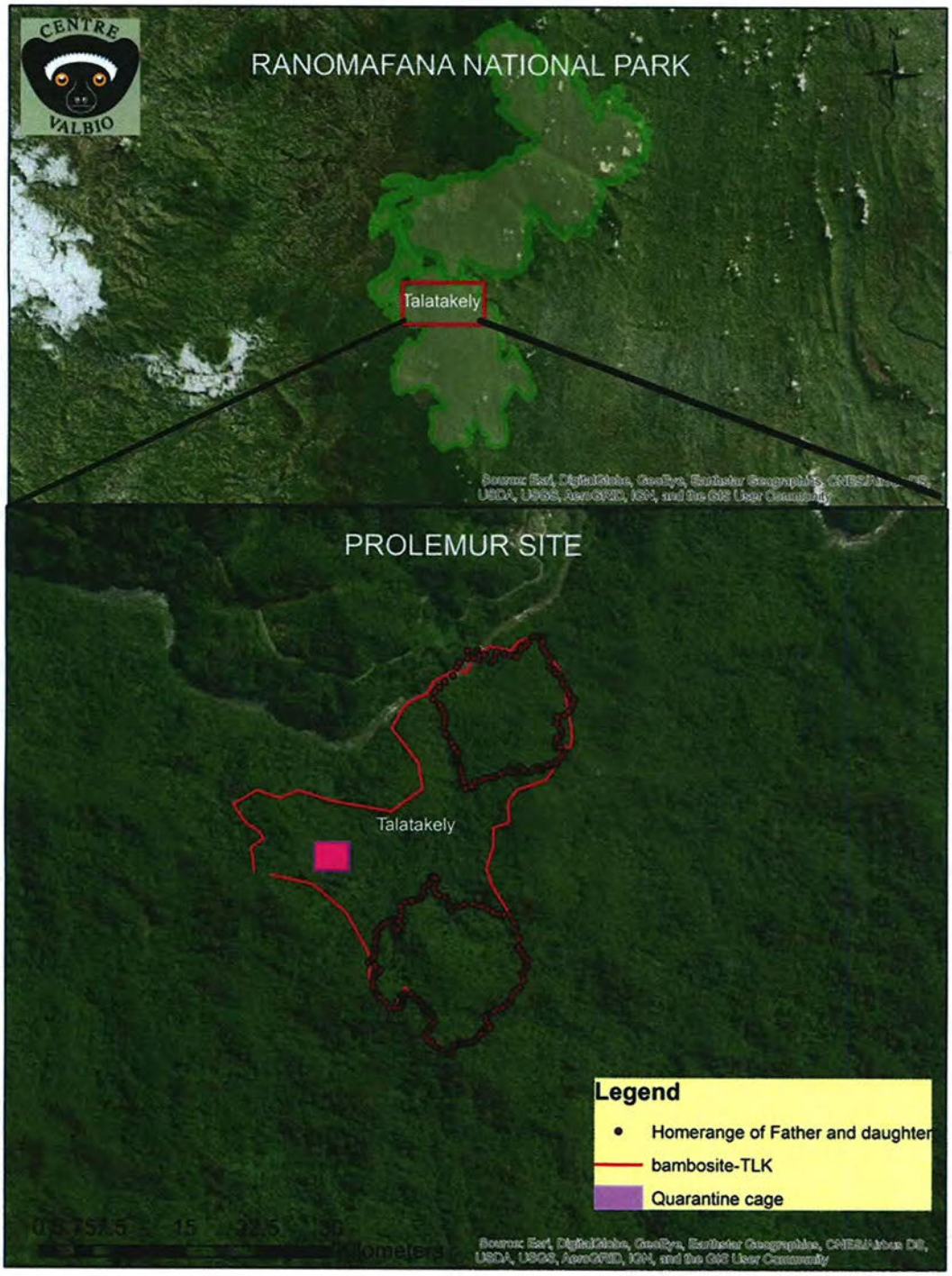
d. Relâche et suivi:

Tous les animaux capturés auront des colliers et l'équipe de techniciens du CVB et les chercheurs suivront les lémuriens résidents et relâchés sans arrêt pendant au moins un mois. Tous les comportements d'alimentation, agression, toilettages réciproques et repos seront enregistrés. S'il y a des problèmes, l'équipe de capture sera disponible pour les capturer pour un bilan de santé.
Les animaux seront libérés lorsque nous sommes surs que les animaux résidents approchent la cage et on pourrait observer les interactions.
Entre 14 à 30 jours, quand les animaux seront habitués à l'enclos, un enregistrement de vocalisation des animaux dans le territoire de Talatakely sera envoyé le matin. Les observateurs vont enregistrer tous les comportements de ces animaux à l'entente de cette vocalisation. D'autre part, les individus du territoire de Talatakely seront également suivis et leurs comportements à l'entente de la vocalisation seront également enregistrés.
Nous espérons que le groupe de Talatakely sera par la suite attiré par la vocalisation et va venir vers l'enclos et voir les nouveaux individus. Cette période de quarantaine de deux semaines donnera aux deux groupes, résidents et transférés, une occasion de se rencontrer et d'interagir avant la libération du nouveau groupe.
Une fois libérés, nous suivrons leurs mouvements à l'aide des colliers de repérage. Simultanément, nous suivrons également le groupe résident.

Description et localisation de l'enclos

L'enclos sera construit à Talatakely, dans le parc national de Ranomafana, à l'extérieur du domaine vital des deux RNP Prolemur simus et dans la zone de couverture en bambou. L'enceinte mesurera 15 m sur 15 m et 4 m de haut. Il sera construit par des bois et des grillages. L'enclos sera situé dans un endroit dense en bambou et éloigné des pistes de Talatakely afin que les lémuriens ne soient pas dérangés par les touristes.

Mise en quarantaine : Les animaux devront être relâchés quand leurs états de santé seront vérifiés et confirmés par le vétérinaire. En effet, la relâche peut se faire quand l'animal a une bonne perception pour comprendre les environs et les prédateurs. Les animaux devront être relâchés le plus vite que possible dans des endroits préalablement étudié. En effet, *Prolemur simus* est très sensible aux différents stress et le maintien de l'animal dans un enclos pour la quarantaine ne ferait qu'augmenter leurs stress, Selon les directives de l'UICN relatives aux bonnes pratiques, le lémurien peuvent rester au minimum 14 jours dans la cage et au maximum 30 jours. Ils seront nourrit deux fois par jour.



Le suivi sera donc effectué à l'aide des émetteurs et en adoptant la méthode d'Altman (1974). Pendant les observations, les activités générales de l'animal sont enregistrées toutes les cinq minutes à savoir la locomotion, le déplacement, l'alimentation (espèce de plante nourricière, partie consommée etc...) et autres (la communication, l'affiliation, l'accouplement, agression....). Les coordonnées des points le long du trajet de l'animal sont enregistrés pour estimer la taille de son territoire et identifier les lieux d'alimentation ou de dortoir etc...
De temps en temps, les espèces compétitrices ou prédatrices rencontrées sont aussi notées (nom de l'espèce, coordonnées géographiques des lieux de rencontre) pour estimer la pression des prédateurs.

Le suivi écologique se fait au moins 5 jours par semaine jusqu'au moment ou la vie des groupes se stabilise. C'est à dire quand le groupe commence à avoir un territoire défini ou adhérer à un groupe local.
Quand la vie des groupes relâchés est stable avec un territoire plus ou moins défini, on procède à l'étude de son nouveau microhabitat pour savoir sa structure et sa composition floristique. Les échantillonnages se feront dans 4 plots botaniques de 20mx50m dans des altitudes différents (crête, versant, bas fond). Dans chaque plot botanique on identifie le nom et la famille des espèces ligneuses plus de 5cm de DHP (diamètre à hauteur de poitrine), l'espèce de bambous et le volume de canopée d'arbre plus de 5cm de DHP et les caractéristiques abiotiques du microhabitat.
Une évaluation de réintroduction serait effectuée à la fin de la première année de suivi., Les analyses génétiques des données collectées seraient effectuées dès que possible et les résultats seront également utilisés pour l'évaluation de la réintroduction.
Mise en place d'un système d'éducation après relâche sur le projet de réintroduction pour éviter des mauvaises intentions des quelques personnes riveraines.

En effet, la réussite de ce projet sera mesurée par les critères suivants:
1) Les individus introduits dans ce groupe de Talatakely seront acceptés dans le groupe.
2) Des bébés seront nés dans le groupe dans les trois années qui suivent la réintroduction
3) Le groupe du fragment a IVATO ne sera pas affecté par le manque d'individus

Suivi à long terme :
Des suivis réguliers et continus des animaux à long terme seront effectués par le Centre ValBio et de Madagascar National Parks, Ranomafana. En effet, les suivis devraient être effectués selon les méthodologies scientifiques standards du suivi écologique, au moins une fois par an pendant une période de 2 – 3 mois de préférence pendant la saison de la reproduction jusqu'à la naissance.
Les colliers seront remplacés après 18mois et une évaluation de l'état de santé des animaux sera effectué pendant ce remplacement du collier.
Pour le suivi à long terme, chaque institution aura des rôles et responsabilités bien définis:
Le Centre ValBio avec le chef de volet de conservation et Recherche de Madagascar National Parks assureront la coordination technique et scientifique et la réalisation du suivi.
Madagascar National Parks, Ranomafana facilitera l'obtention des permis et démarches nécessaires pour la conduite de ce suivi auprès du Ministère de l'Environnement et du Développement Durable
Les dépenses pendant ces suivis seront discutées et partagés entre les partenaires.

RESULTATS ATTENDUS

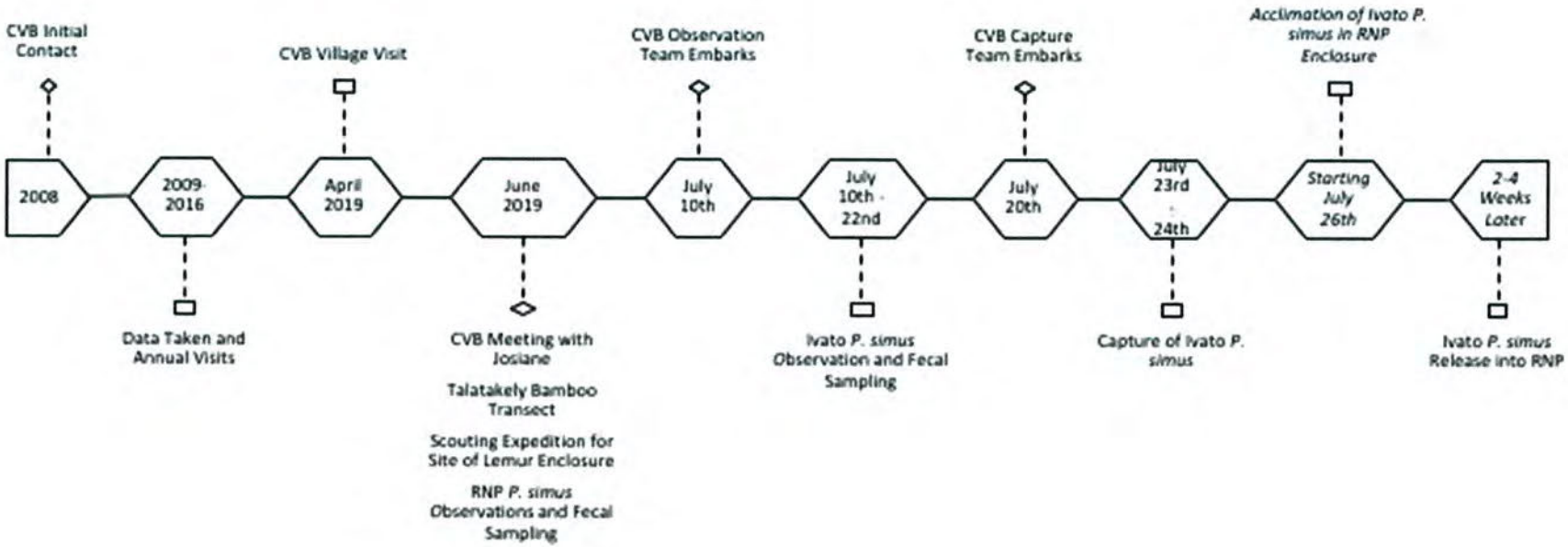
- Rapport de tout le processus de la réintroduction pour le Ministère de l'Environnement et du Développement Durable et le Madagascar National Parks
- Accomplissement de la translocation et réintroduction d'une espèce en danger en dehors d'aires protégées vers une aire protégée
- Augmentation de la variation génétique d'un groupe de *Prolemur simus* habitué dans un Parc National
- Création d'un modèle de réintroduction pour les lémuriens de Madagascar dans le futur
- Soumission de publication scientifique sur ce projet de réintroduction
- Population riveraine éduquée
- Communauté internationale dans le domaine de l'environnement et de la conservation informés sur cette réintroduction
- Groupe de *Prolemur simus* stable dans l'aire protégée du Parc National de Ranomafana
- Société sans problèmes après la capture

Liste des participants :

- Patricia Wright – Principal Investigateur
- Bekah Weatherington – Responsable des collectes et analyse des échantillons
- Ezzeldin Enan- Responsable des collectes et analyse des échantillons
- Fidy Rasambainarivo- Vétérinaire
- Haja Rakotondrainibe - Vétérinaire

- Jake Krauss – étudiant assistant pour le suivi de comportement
- Amanda Du Bour – étudiant assistant pour le suivi du régime alimentaire
- Ryan Rothman - assistant pour le suivi de comportement et régime alimentaire
- Lianne Woudstra - assistant pour le suivi de comportement et régime alimentaire
- Thomas Kelly - étudiant assistant pour le suivi de comportement
- Tobias Gräßle- Vétérinaire
- Sina Feyer- Vétérinaire
- Heninkaja Rasoaviarimanana – étudiante assistant pour le suivi du régime alimentaire
- 2 représentants MNP – supervision
- Représentants MEDD – supervision
- Pascal Rabeson - supervision
- Velotsara Jean Baptiste – technicien capture
- Dina Andrianoely – technicien recherche
- Georges Razafindrakoto- technicien capture
- Mamitina Velonabison - technicien capture
- Georges Rene Rakotonirina – technician suivi
- Remi Rakotovao – technicien suivi
- Dominique Razafindraibe- technicien suivi
- Randrianasolo Laurent – technicien suivi
- Zakamanana Francois – technicien suivi comportement

Chronogramme des activités pour la reintroduction			
Date	**Activités**	**Intervenants**	**Observations**
16/11/2018	Visite à Ivato et demande d'autorisation a l'autorité locale et la communauté locale pour la capture de simus Suivi de 4 agents CVB et 4 guides locaux des simus a Ivato	CVB, CANTONNEMENT, Ray aman-Dreny Ivato	accompli
05/06/2019	Proposition de réintroduction rendue au Directeur du Parc	MNP, CVB	accompli
08/06/2019	8/6/2019 Visite site de réception (Talatakely)	MNP, CVB	accompli
15/06/2019	Emplacement cage a Talatakely	CVB, MNP	En cours
17/06/2019	Reunion avec Mamy DG MNP Tana	CVB MNP	En cours
19/06/2019	Réunion comité préparatoire	DREDD, CIREDD, Chef Cantonnement, MNP, CVB, Maires, Guides touristiques	En cours
19/06/2019	Envoi demande de capture et documents nécessaires à Manakara avant l'envoi à Antananarivo	DREDD, CIREDD, Chef Cantonnement, MNP, CVB	En cours
04/07/2019	Capture simus a Ivato et mis en enclos à Talatakely. Examen de santé et pose de collier au Centre ValBio	équipe de capture, MNP, CVB, Rep. autorité, Primatologue, Rep. com. Base	En cours
07/07/2019	Simus en enclos a Talatakely	équipe de capture, MNP, CVB, Rep. autorité, Primatologue, Rep. com. Base	En cours
21/07/2019-04/08/2019	Relâche simus dans la foret selon son état de santé	équipe de capture, MNP, CVB, Rep. autorité, Primatologue, Rep. com. Base	En cours
After 04/08/2019	Réunion d'évaluation du projet reintroduction du comite de reintroduction	DREDD, CIREF, CANFORET, MNP, CVB, autorité locale	En cours


CVB Initial Contact
2008
2009-2016
Data Taken and Annual Visits
CVB Village Visit
April 2019
June 2019
CVB Meeting with Josiane
Talatakely Bamboo Transect
Scouting Expedition for Site of Lemur Enclosure
RNP P. simus Observations and Fecal Sampling
CVB Observation Team Embarks
July 10th
July 10th - 22nd
Ivato P. simus Observation and Fecal Sampling
CVB Capture Team Embarks
July 20th
July 23rd - 24th
Capture of Ivato P. simus
Acclimation of Ivato P. simus in RNP Enclosure
Starting July 26th
2-4 Weeks Later
Ivato P. simus Release into RNP

Conclusions et recommandations

En conclusion, toutes les conditions pour la réintroduction sont remplies et il est évident que la translocation de quelques individus de Prolemur simus de la forêt d'Ivato et sa réintroduction vers la forêt de Talatakely pour sauvegarder les deux individus restant s'avère très important pour la conservation de cette espèce et l'avenir du tourisme du site de Ranomafana. Après cette réintroduction, la diversité génétique sera assurée pour la continuité de la viabilité de cette population d'aire protégée. Nous sommes convaincus à la réussite de ce projet de réintroduction et espérons que cet exemple sera un modèle de réintroduction d'une espèce en danger tant au niveau national qu'international.

Références bibliographique

1) Altman J. 1974. Observational study of sampling methods. Behavior 49:227–267.

2) Beck B. 2017. Unwitting Travelers: A History of Primate Reintroduction

3) Fausser, J.L.; Prosper, P.; Rumpler, Y. 2002. Relationships inside the genus Hapalemur based on mitochondrial DNA sequences. Lemur News 7: 25-26.

4) Gillespie, T. R., Nunn, C. L., & Leendertz, F. H. (2008). Integrative approaches to the study of primate infectious disease: Implications for biodiversity conservation and global health. American Journal of Physical Anthropology,137(S47), 53-69. doi:10.1002/ajpa.20949

5) Godfrey, L.R.; Simons, E.L.; Jungers, W.L.; DeBlieux, D.D.; Chatrath, P.S. 2004. New discovery of subfossil Hapalemur simus, the greater bamboo lemur, in western Madagascar.Lemur News 9.

6) Mittermeier, R.A.; Tattersall, I.; Konstant, W.R.; Meyers, D.M.; Mast, R.B. 1994. Lemurs of Madagascar. Conservation International, Washington, D.C.

7) Tan, C.L. 1999. Group composition, home range size, and diet of three sympatric bamboo lemur species (genus Hapalemur) in Ranomafana National Park, Madagascar.Int. J. Primatol. 20: 547-566.

8) Mittermeier, R.A ; Valladares-Pádua Cláudio, Anthony B. Rylands, Ardith A. Eudey, Thomas M. Butynski, Jörg U. Ganzhorn, Rebecca Kormos, John M. Aguiar, Sally Walker.2005. The World's 25 Most Endangered Primates 2004–2006

9) IUCN (2003). "Newsletter of the re-introduction specialists group IUCN's species survival commission (SSC)." re-introduction news 23(ISSN 1560-3709): 46.

10) IUCN (2005). "Newsletter of the re-introduction specialists group IUCN's species survival commission (SSC)." Re-introduction news 24: 50.

11) IUCN (2006). "Newsletter of the re-introduction specialists group IUCN's species survival commission (SSC)." re-introduction news 25(ISSN 1560-3709): 52.

FARITANY FIANARANTSO
FARITRA ATSIMOANDRANO
DISTRIKA VONDROZO
KAOMININA AMBANIVOHITRA
IVATO

REPOBLIKAN' I MADAGASIKARA
Fitiavana - Tanindrazana - Fandrosoana

NY P.D.S. ETO IVATO

Ho an'

Andriamatoa LEHIBEN'NY ALA SY NY RANO AO

-V O N D R O Z O-

Antony: Tatitra Fanolorana indray ny toerana fitahirizana VARIKA ao Mahasoa.

Tompoko,

Voninahitra lehibe ho anay P.D.S. Ivato, no ahazoanay manao izao tatitra izao aty aminareo hoho izao antony manaraka izao:-

Ny Zoma 07 desambra 2018, dia nivory ny Fokonolona anatin'ireto Tanàna manaraka ireto:

-Ivato - Emita - Ambalateny , mahakasika ny fiompiana Varika any Mahasoa.

Tanaka fa mbola manolotra indray ny Tany any mahasoa . (Centre de Conservention VALBIO ny any Mahasoa).

Raiso tompoko ny haja sy ny Voninahitra atolotray anao.

Ivato, 07 desambra 2018

LE PREMIER VICE
PRESIDENT DE LA DELEGATION
SPECIALE

MARONSY Sylvain Fiacre
OPERATEUR

Vu et lu accordé
Le Chef Cantonnement de l'Environnement
de l'Ecologie et des Forêts

FARITANY:FIANARANTSOA
FARITRA:ATSIMO ATSINANANA
DISTRIKAN'I VONDROZO
KAOMININA : I V A T O
FOKONTANY : I V A T O

REPOBLIKAN'I MADAGASIKARA
Fitiavana - Tanindrazana - Fandrosoana

Ny Fokonolona eto Ivato sy Emita ary Ambalateny, Kaominina Ivato. Distrikan'i Vondrozo.

Antony: Fanolorana Tany itoeran'ny VARIKA ao Mahasoa-Ivato.

Ho an'

Andriamatoa LEHIBEN'NY ALA SY NY RANO AO
- V O N D R O Z O -

Ampandalovina amin'Andriamatoa PDS. Ivato

Tompoko,

Voninahitra lehibe ho anay Fokonolona voalaza anarana etsy ambony no ahazoanay mandefa an'ity Taratasy Fanolorana Tany itoeran'ny varika ao Mahasoa io noho izao antony manaraka izao:-

Ny Zoma faha 07 desambra 2018, dia nivory teto Ivato ny Fokonolona telo Tanànana:-Ivato - Emita - Ambalateny mahakasika ny fiompiana Varika eny Mahasoa;

dia tapaka tamin'izany fotoana izany, dia mbola manolotra an'io toerana misy an'io Varika io indray izahay mpivory telo Tanàna izay voalaza etsy ambony.

Raiso Andriamatoa ny haja amam-boninahitra atolotray anao.

Sonia :

Ny Fokonolona
Rabemahafaly
Botomaniet
TABILA Jean
Randriamanana Ida Evariste
RANDRIAMIADANA Philemont
Benoavy Auguste
BOTOMISEBIRA | OMBRI
MAHIMBA | VENOT
WILIAM | Rekoty Thermont
BOTOLEO | Felet
VELOTO | Gostin
BOTOMADY | Kaka
LETSOA

LE PREMIER VICE PRESIDENT DE LA DELEGATION SPECIALE
MARDASY Sylvain Fidelis
OPERATEUR

Vu et [illegible] accorde

Le Chef Cantonnement de l'E[illegible] de l'Ecologie [illegible]

LEMURS HANDLING SHEET (VALBIO CENTER _ JULY 2019)			
DATE:	RESEARCHERS NAME:		
LEMURS IDENTIFICATION			
ID		MICROCHIP	
SPECIES		SEX	
GROUPE		AGE EST	
INDIVIDUAL AFFILIATION		NEW RADIO	
DISTINGUISHING MARK		NEW NYLON	
IMMOBILIZATION INFO			
DATE/HOUR		ZONE	
CAPTURE METHOD		GPS	
DRUG		DOSE	

PHYSICAL EXAM				
	NOR	ABN	COMMENTS	
BODY CONDITION			OVERWEIGHT / GOOD /	
ORAL CAVITY			BLOOD / OBSTRUCTION	
MUCOUS COLOR/CRT				
TEETH			MISSING / BROKEN	
MOLAR WEAR			NONE / MILD / MODERATE / SEVERE	
TOOTH COMBWEAR			NONE / MILD / MODERATE / SEVERE	
EYES			DULL / DISCHARGE / INFLAMMED / CLOSED	
EARS			INJURED / DISCHARGE / PARASITES / DEBRIS /	

NOSE			DISCHARGE / DRY / SORES / LACERATION	
HAIRCOAT			THINNING / UN-GROOMED	
SKIN			SORES / WOUNDS	
TICKS / LOCATION:			FEW / MOD / MANY	
MITES / LOCATION:			FEW / MOD / MANY	
LICE / LOCATION			FEW / MOD / MANY	
FLY /LOCATION			FEW / MOD / MANY	
ABDOMINAL PALP				
LYMPH NODES				
MUSCULOSKELETAL			LIMPING / DECREASED	
CARDIO			MURMUR / ARRHYTHMIA	
RESP			CRACKLES / WHEEZES	
NIPPLES				
GENITAL/URINARY:				
SC FLUIDS				

SAMPLE COLLECTION				
	Y	N	QUANTITY	COMMENT
BLOOD (EDTA-purple top)				
BLOOD (heparin-green top)				
DENTAL PHOTOS				
LESION PHOTOS				
TISSUE SAMPLE				
URINE (red top)				
BREATHE				
SWABS				SCENT GLANDS/GENITAL/RECTAL/OCULAR/NASAL/ORAL
ECTOPARASITES (ethanol)				TICKS / MITES / LICE / FLIES
FECES (formalin and PVA)				
HAIR				
ID PHOTOS				
FINGERPRINTS				

MEASUREMENTS			
GENERAL BODY			
Weight (Kg)		Biceps circ. (cm)	
Head crown(cm)		Chest circ. (cm)	
Body length (cm)		Thigh circ. (cm)	
Tail length (cm)		Nipples (cm)	
FORELIMB (length)		HINDLLIMB (length)	
Thumb (cm)		Thumb (cm)	
Longest digit (cm)		Longestdigit (cm)	

Hand (cm)		Foot (cm)	
Ulna/Radius ((cm)		Tibia (cm)	
Humérus (cm)		Fémur (cm)	
CANINE		MUZZLE	
Upper (mm)		Length (mm)	
Lower (mm)		Width (mm)	
EAR			
Length (mm)		Width (mm)	
TESTES			
Right length (mm)		Left length (mm)	
Right width (mm)		Left width (mm)	
GENERAL COMMENTS			

ANESTHESIA				
ANAESTHESIC SUPPLEMENT			RECOVERY	
TIME	DRUG	DOSE	TIME	COMMENT

VITAL SIGNS			
TIME	HEART RATE (BPM)	RESP RATE (BPM)	TEMP (F / C)

Blood

Green tube:

PCV (%)_____ Glucose _____

TP _____ Plasma appearance _____

Purple tube: CBC

White Blood Cell Estimate: on 40X or 50X on the monolayer

Field 1	Field 2	Field 3	Field 4	Field 5	Average

Avg. X obj^2= _____

White Blood Cell Differential: 100X oil immersion on the monolayer

	Cell Count	Differential Count (%)	Absolute count (x tWBC)		Cell Count	Differential Count (%)	Absolute count (x tWBC)
Segmented neutrophils				Monocytes			
Band neutrophils				Basophils			
Lymphocytes				Other			
Eosinophils				Total	100	-	-

Platelet smear estimate: 100X oil immersion on the monolayer

Field 1	Field 2	Field 3	Field 4	Field 5	Average

Avg X 15,000 = _____ (180-225,000/uL)
Azostix (BUN):_____
Hemoparasites:
WBC morphology: RBC morphology:

Urine
Color: _____ Clarity: _____ Refractometer- Urine Specific Gravity: _____
Dipstick results:

Leukocytes	Nitrites	Urobilinogen	Protein	pH	Blood	USG	Ketones	Bilirubin	Glu

Sediment Exam:

Bloodwork Protocol for Lemur Translocation- Bekah Weatherington
Purple top tube (EDTA anticoagulant): yields plasma, contains fibrinogen and clotting factors

- Mix well using inversion
- Promptly make 2 blood smears
- Let air dry
- Dip quick stain
    - 5 dips in each stain
    - Rinse with water
- Let air dry
- Examine under microscope
    - CBC
        - WBC estimate
            - Use 50X on the monolayer
            - Avg in 10 fields X 2,500 (or the objective squared, ie multiply by 1600 if on 40X)
        - WBC differential
            - Use 40X or 100X oil immersion
            - Count 100 total
        - Platelet estimate
            - Use 100X
            - Avg in 5 fields X 15,000
            - Tip: normally see 12-15 platelets/hpf, equating 180-225,000 platelets/microL
        - Note WBC and RBC morphology
    - Hemoparasites

Green top tube (heparin anticoagulant): yields plasma

- PCV and TP
    - Load 2 hematocrit tubes
    - Spin down- 5 min on hematocrit centrifuge
    - Measure PCV using reader card
    - Break tube and measure TP on refractometer
- Glucose

- Place strip in glucometer
- Touch end to drop of blood (follow glucometer manual)

- CHEM panel- if red top tubes unavailable, only if have the ability to freeze (liquid nitrogen)
  - Need to fill 2 green microtainer tubes
  - Centrifuge
  - Pipette off 1ml plasma
  - Freeze to store until able to run CHEM panel in lab

Red top tube (no anticoagulant, clotted blood): yields serum, no fibrinogen, some clotting factors

- CHEM panel- red top tube ideal, only if have the ability to freeze (liquid nitrogen)
  - Allow one hour to clot
  - Centrifuge
  - Pipette off 1ml serum into clean tube
  - Freeze to store until able to run CHEM panel in lab

Protocol for Sample Collection During Field Immobilization

We are following the collection protocol recommended in Gillespie et al 2008. These samples will be collected during field immobilization.

- Each
  - Any observed abnormalities
  - Examination of the oral cavity, eyes, and ears
  - Auscultation of heart and lungs
  - Recording of body temperature and respiratory rate
- External measurements including:
  - Weight
  - Body length
  - Tail length
  - Girth (At widest point)
  - Neck circumference
  - Foreleg / Forefoot length
  - Hindleg / Hindfoot length
- The dentition of each animal will be digitally photographed
- The Following samples will also be collected as recommended in Gillespie et al. 2008:
  - Blood: 10–20 ml, not to exceed 1% of body weight, should be collected from the femoral vein after disinfecting the site with alcohol. Appropriate sized needles must be selected to prevent trauma and venipuncture site must be monitored briefly for hematoma formation. Today, collection of EDTA blood is recommended since this allows analyses of whole blood, plasma and ''Buffy Coat''. A drop of the blood collected should be used for thin and thick smears, then the collection tube can either be centrifuged to separate plasma from cells or (if no centrifuge is available) EDTA tubes can be stored standing until the plasma has separated from the cell-rich fraction. Once separation has occurred, plasma and cells should be stored separately frozen (or if not possible dried).
  - Hair: Several pinches of hair pulled from the base of the tail should be collected as a source of genetic material and for detection of integumentary pathogens. A glove or instrument must be used to avoid contamination with human tissue.
  - Mouth swabs: Inside of the cheeks should be swabbed for detection of oral pathogens.

- Tracheal swabs: Swabs of the trachea should be collected for detection of respiratory pathogens. This is a challenging procedure for many species and requires some level of expertise. This procedure also presents a small but real risk of an animal bite, even in an anesthetized animal, due to jaw reflexes.

- Animal will receive a complete physical examination including:
  - Penile/vaginal swabs: Swabs of the penis or vagina should be collected for detection of sexually transmit- ted pathogens.
  - Rectal/fecal samples: Rectal swabs and fecal samples should be collected for detection of gastrointestinal pathogens.
  - Ocular swabs: An eye should be swabbed for the detection of ocular pathogens. This should be done with care to avoid corneal trauma from abrasion.
  - Nasal swabs: Swabs of the nose should be collected for detection of respiratory pathogens.
  - Ectoparasites: The hair and skin of the animals should be examined with fine-toothed combs. Any ectoparasites (e.g. lice, ticks, fleas) thus obtained should be saved for identification in 70% ethanol.
- These samples will be refrigerated, stored in RNALater, or dehydrated for future pathogen analysis

Collection of Fecal Samples for Gastrointestinal Parasite Analysis

- Each Fecal sample will be split into 10% Buffered Formalin for Helminths and Polyvinyl Alcohol for Protozoa
- Fecal samples will be examined macroscopically for consistency, blood, mucus, or visible larvae. Observed helminths will be preserved for identification
  - Nematodes will be washed 3 times with 1% saline solution. They will then be transferred to hot 70% Ethanol or 5% formalin (70-80°C). After cooling, they will then be transferred to a cool solution of the same kind.
  - Cestodes (Tapeworms) will be fixed in 5-10% formalin between two pieces of glass. They can also be dipped repeatedly while suspended with forceps to prevent contraction
  - Trematodes (Flukes) will be washed with 1% saline while being vigorously shaken. Saline will then be replaced by 5-10% formalin while still shaking.
- Using a wooden applicator stick, a 2g fecal sample will be collected from inside the bolus to prevent contaminants and placed in a 15mL centrifuge tube. (6g for 50mL tubes). The tubes will then be vigorously shaken.
- Tubes will be properly labeled with the Individual's ID number. Notations will be made containing observed Age, Sex, GPS coordinate of sample, Date & Time of collection.

The table below has an overview of the information collected per sample. Daily measurements of temperature, humidity, precipitation, etc. will be taken and noted during sample collection.

- If possible, samples will be stored in a fridge 4°C. If not, they will be kept in a cool dark place until laboratory analysis.
- If possible, samples will be duplicated and placed in RNA later for molecular confirmation of parasite identities.

Sample Number	Example ID# 1234	1	2	3
Preservative F=10% buffered formalin P=Polyvinyl Alchohol E=Ethanol	F			
Name	Prolemur bekah			
Date	06/03/2019			
Time of Defecation	14:23			

Time of Sample Collection	14:24			
GPS Coordinates of Sample collection	-21.237848, 47.435306			
Age	3			
Sex	Female			
Macro-observation	Blood in feces; Nematode ID:1234; Hair in feces			
Consistency of Feces H=Hard; M=Medium; S=Soft; F=Fluid	S			
Comments	Lactating; Sneezing; Lethargic etc.			
Stored at R= Room temp; F=4°C Fridge	R			
Collected By	Ezzeldin Enan			
Date of Laboratory Analysis	06/04/2019			

Fecal Floatation

- Fecal Floatation will be done using Sheathers sucrose solution with a specific gravity of 1.27 as well as Zinc Sulfate (ZnSO4) with a specific gravity of 1.18
- 1-2g of feces will be placed in a 15mL centrifuge tube and ~10mL distilled water will be added. The mixture will then be homogenized using a wooden applicator stick.
- They will then be centrifuged at 1800 rpm for 10min before pouring off the supernatant and resuspending it in Sheathers sucrose solution or zinc sulfate
- The additional solution will be added to the tube until an inverse meniscus and a cover slide will be placed on top and centrifuged for another 10 minutes at 1800 rpm
- After centrifugation, the coverslips were placed on prelabeled microscope slides with the Sample ID number.
- Slides will then be examined systematically for parasites. Lugol's Iodine Solution will be used as a simple stain to facilitate identification.
- Parasite egg, larvae, and cyst morphology will be measured using a calibrated ocular micrometer
- Representatives will be Photographed

Fecal Sedimentation

- Fecal samples will be suspended in 40 mL of diluted soapy water (Non-toxic biodegradable soap)
- The suspension will be filtered through a double layer of gauze presoaked in the sedimentation solution. The remaining pellet and gauze will be washed down using an additional ~5-10mL of sedimentation solution, into a centrifuge tube.
- After centrifuging for 10 minutes, the supernatant will be decanted and the sediment resuspended in the sedimentation solution. This will be repeated until the supernatant is clear.
- A few drops of the sediment will then be placed on a pre labeled microscope slide and covered with two coverslips.
- Slides will then be examined systematically for parasites. Lugol's Iodine Solution will be used as a simple stain to facilitate identification.
- Parasite egg, larvae, and cyst morphology will be measured using a calibrated ocular micrometer
- Representatives will be Photographed

Opportunistic Necropsy

- A rectal/fecal sample will be collected for later analysis
- After ligating and removing the stomach and intestines, string will be used to tie the stomach, small intestine, large intestine, and colon.
- Each section will be cut open and contents collected and washed separately.
- The walls of the stomach and intestine will be thoroughly washed and any larvae collected.
- The wash will be filtered through 0.15mm aperture for recovering adult worms, and then 0.038mm for recovering immature worms. The screen will then be repeatedly washed until it runs clear.
- The surface of stomach, intestines etc. will then be carefully examined with a dissecting microscope for parasites that remain attached.

Trichrome Stain Protocol for Parasitology PVA-Fixed Specimen

- Allow specimen to fix in LV-PVA (Low-Viscosity Polyvinyl Alcohol) for at least 30 minutes. Mix thoroughly with applicator sticks.
- Pour a small amount of the LV-PVA fixed material onto a paper towel, and stand for 3 minutes to absorb excess PVA.
- Using an applicator stick, apply (Do not smear) some of the material from the paper towel onto microscope slides.
- Dry the slides overnight at room temperature
    - Or at 37°C in an incubator for several hours
    - Or on a slide warmer for several hours

Accelerated drying is not recommended due to morphology distortions ○ Do not proceed until slides are completely dry. Once the slides have dried completely, proceed with the following staining protocol.

70% Ethanol Plus Iodine Stock Solution Procedure

1. Prepare a stock solution by adding iodine crystals to 70% alcohol until a dark solution is obtained (1-2g/100mL)
2. To use, Dilute the stock solution with 70% alcohol until a dark red-brown or strong tea color is obtained

90% Ethanol Plus Acetic Acid

1. Mix the Following:
    1. 90% Ethanol (99.5mL)
    2. Acetic acid (Glacial) (0.5mL)

Stain Procedure

70% Ethanol plus Iodine	5-10 minutes
70% Ethanol	5 minutes
10% Ethanol	3 minutes
Trichrome Stain	10 minutes
90% Ethanol plus acetic acid (Drain slides immediately and proceed)	1-3 seconds

100% Ethanol	Dip 2-3 times
100% Ethanol	3 minutes
100% Ethanol	3 minutes
Xylene	5-10 minutes
Xylene	5-10 minutes
Mount with a coverslip and Mounting Medium	

Results:

Protozoan trophozoites and cysts will be readily seen. *Entamoeba histolytica* w ill have a blue-green cytoplasm and cysts will also appear blue-green. *Entamoeba Coli* cysts will appear blue-green with a purple tint. Helminth eggs and larvae (wet smears from concentration are recommended) retain stain and will appear red-purple. Yeast may be identified and will appear green. Human cells such as RBC's will appear red. Karyosomes of nuclei, chromatoid bodies, and chromatin material appear red-purple.

Collecting Swabs of Scent Glands

Items needed:

- Small container or Ziploc bag (to hold sterile materials)
- Cotton-swabs (sterile)
- 15mL falcon tubes (sterile)
- Scissors (clean)
- Alcohol wipes (to wipe down scissors before every use)
- Examination gloves
- Cooler with ice or ice packs (to keep samples cool until freezing)

Procedure:

1. Put on examination gloves for sampling. If gloves become contaminated, you should replace them.
2. Use an alcohol wipe to sterilize scissors (wipe down the blades of the scissors).
3. Cut a sterile cotton swab in half.
4. Carefully remove the sterile cotton swab from the packaging by pulling out the handle.
5. Obtain sample by rubbing the swab across the glandular region three times.
6. Open a sterile falcon tube and immediately place the swab (tip in first) into the tube. Close the lid of the tube immediately.
7. Place the sample into the cooler and transport it back to lab.
8. Store the samples in a freezer (ideally -80 C).

Whenever possible, it helps if two people work together to prepare the materials and obtain the sample. Be sure to always use gloves to ensure that materials are free of DNA contamination.

***Prolemur simus* Breath Sampling Protocol**

To sample volatile organic compounds (VOCs) that are exhalants from bamboo lemurs, we will use a method developed (Zohdy, Starkey, Blagburn patent pending) which has been optimized for the detection of breath compounds only and not environmental surroundings.

Equipment:

- Teflon coated tubing
- Modified gas pump with in/out valve
- Modified coated flexfoil bags with sealable septum -Sharpie for labeling
- Scissors
- Rubber gloves

Sampling:

- The sampler should put on a fresh pair of rubber/nitrile gloves per sample bag -Cut two 3-4 inch long pieces of tubing
- Attach one to a sampling bag and "out" valve on pump
- Attach the other piece of tubing to the "in" valve
- Turn on pump and hold tubing connected to "in" valve ~2 inches away from the lemur's nostrils to capture exhalant. Keep pump on for 1-2 minutes or until bag is 1/2 to 3/4 full
- Turn off pump
- Close valve attached to sample bag until sealed. Make sure there is no leak and that it is sealed shut
- Label bag with date, location (GPS coordinate), collector's name, species name, individual ID, sex, body mass, type of ectoparasites found, quantification of ectoparasites (true number or infestation level: none, low, medium, heavy)
- If possible, please collect representative ectoparasites in ethanol labeled with individual ID

As a negative control, collect a sample bag using the same methods listed above, but collect ambient air from the location in which you sampled the lemur (the forest, campsite, bamboo forest, ag site, etc.). Label the bag in the same way. This is very important for distinguishing breath VOCs from others. A negative control bag should be collected for each location where lemurs are captured.

SOMMAIRE

Patricia Chapple Wright
[redacted]

RESEARCH INTERESTS

Tropical biology:

- Effects of climate change on rainforests
- Ecosystem dynamics (i.e. seed dispersal, pollination, etc.)
- Predator-prey relationships
- Exploration

Conservation:

- Drivers for successful community-based conservation
- Use of technology to monitor anthropogenic activities
- Translating science to general audiences through education and public awareness
- Social and biological framework for the creation of protected areas
- Wildlife and protected area policy

Primates (behavior, ecology, and biology), and ecosystem health:

- Genetic consequences of dispersal in primates
- Monogamy, parental care, infant development, and female dominance in primates
- Demography of lemurs
- Effects of predation and parasites on primate biology
- Effects of aging on wild primate behavior
- Primate genomics for disease understanding
- Microbiome dynamics in wild primates
- Diseases in primates and humans and their interface.
- Understanding of effects of land-use changes on disease vectors

CURRENT POSITIONS

- Distinguished Professor in the Department of Anthropology at the State University of New York at Stony Brook ("SBU")
- Founder and Executive Director of the Institute for the Conservation of Tropical Environments (ICTE), SBU
- Founder and Executive Director of Centre ValBio Research station, Ranomafana National Park in Madagascar

PREVIOUS POSITIONS

• Full Professor, SBU, Department of Anthropology	1996-2014
• Associate Professor, SBU, Department of Anthropology	1991-1996
• Assistant Professor, Department of Biological Anthropology, Duke University	1986-1990

EDUCATION

POSTGRADUATE	City University of New York, USA	PhD in Anthropology (1985)
UNDERGRADUATE	Hood College, Frederick, Md. USA	BA in Biology (1966)

CAREER HIGHLIGHTS (selected)

In 1986, while on an exploratory expedition to Madagascar, along with colleagues I discovered a new species of lemur, the golden bamboo lemur (*Hapalemur Aureus*). When this rain forest, and the future of this new species, were threatened by timber exploitation, my attention turned to conservation. I spearheaded an integrated conservation and development project at Ranomafana that focused on the protection and conservation of endemic flora and fauna as well as rural development, education, and promotion of health services in the park's peripheral zone. In 1991 the Ranomafana National Park was inaugurated. I coordinated the building of the park infrastructure and management, ecotourism development, biodiversity research and monitoring, economic development, health and education within the peripheral zone villages.

In 1997 the Ranomafana National Park management was handed over to the Malagasy Park Service. Since 1997, I have continued to be actively involved in biodiversity research and exploration in Madagascar. My research in Madagascar focuses on the effects of human induced and natural change on Madagascar rainforest, and the effects of pollination and seed dispersal by lemurs and ecosystem dynamics.

FOUNDER OF CENTRE VALBIO RESEARCH STATION

I have spearheaded Centre ValBio ('CVB'), an award-winning, green, sustainable, research station on the edge of the rainforest, with molecular and infectious disease laboratories, high speed internet, and modern facilities. CVB is the hub for programs in biodiversity research, environmental arts, innovative technology, environmental education, health, and reforestation.

AWARD AND HONOURS

2019	St Andrews Prize for the Environment finalist
2018	Distinguished Alumnae Medal of Honor, City University of New York
2017	African Forbes Woman Scientist
2017	Natural World Hero, Natural World Travels
2016-7	Phi Beta Kappa Visiting Scholar
2014	Doctor Honoris Causa, University of Fianarantsoa, Madagascar
2014	Wildlife Conservation Film Festival Lifetime Achievement Award winner
2014	Indianapolis Prize Winner, Indianapolis Zoological Society
2014	Eli Lilly Medal Award winner
2014	Distinguished Professor, State University of NY
2013	Elected to the American Philosophical Society, Philadelphia, NY.
2012	Indianapolis Prize Finalist, Indianapolis Zoological Society
2012	Commandeur National, Medal of Honor of Madagascar
2011	Distinguished Alumna Award from Hood College

2008	Hauptman Woodward Pioneer in Science Medal
2008	Distinguished Primatologist Award from American Society of Primatology
2007	Honoris causa, honorary degree from University of Antananarivo
2007	Ranomafana National Park named UNESCO World Heritage Site
2006	A new species from Kalambatritra Madagascar named *Lepilemur wrightae*
2004	Elected American Association of Science Fellow (AAAS)
2004	Medaille Officier de Madagascar, awarded by the President of Madagascar
2003	Woman of Distinction Award, given by Senator Lavalle
2001	Committee for Research and Exploration, National Geographic Society
1995	"Officier d'Ordre National" National Medal of Honor of Madagascar
1990-7	Integrated Conservation and Development Project (Ranomafana National Park)
1989-94	John D. and Catherine T. MacArthur Fellow
1986	Discovery of Golden Bamboo Lemur *Hapalemur aureus* in Ranomafana Forest Madagascar

PUBLIC OUTREACH

2019	Featured in Science Communications, Leslie Roberts
2019	Featured in Forbes Africa
2019	Letter in Science Communications
2018	Featured in CBS News (Sarah Carter)
2017	Keynote Speaker, Hood College 125th year Anniversary
2017	Keynote Speaker Creating Equilibrium, Equilibrium Productions, CA
2017	Keynote Speaker, Earth Optimism Summit, Smithsonian Institute, Washington DC
2017	Visiting Scholar Series, Phi Beta Kappa,
2016	Keynote Speaker at Ranomafana National Park- 25th Anniversary
2016	Keynote speaker at Duke Lemur Center, 50th Anniversary
2016	Featured in ABC Nightline News (Alex Marquardt)
2015	Featured on CNN's Anthony Bourdain: Parts Unknown
2015	Keynote Speaker, American Philosophical Society Meeting, Philadelphia, PA
2014	Featured in 3D IMAX Film "Island of Lemurs: Madagascar" with Morgan Freeman
2014	Featured in NY Times, Wall Street Journal, Huffington Post, USA Today
2014	Featured in NBC Nightly News, Brian Williams
2014	Reddit, ABC morning talk show
2013	American Museum of Natural History Public Lecture Series, New York, NY
2013	Explorer's Club Lecture Series, New York, NY
2013	Invited Speaker, Conference on Conservation Science, Bangalore, India
2013	Madagascar's Eastern Rainforest Symposium Speaker, Zoo Zurich, Switzerland
2013	Darwin Day Keynote Address, Ohio University
2013	Women in Science Professions Speaker, SUNY Syracuse
2012	Centre ValBio, NamanaBe Hall Inauguration, July 2012
2011-3	Science Advisor featured in IMAX film "Island of Lemurs: MADAGASCAR 3D"
2011	Science Advisor, NHK Science Channel's "Mutant Planet: Lemurs of Madagascar"
2011	Distinguished Lecturer Series, The Huck Institute of Life Science, Penn State
2010	Featured in article for Yale Environment 360 (Kotler, Steven. "As Madagascar is Plundered, A Staunch Defender Fights Back." Yale Environment 360 1 Jul. 2010)
2009	Interviewed in Dan Rather's television program, Dan Rather Reports, NY
2009	Interviewed as expert in TV film for Research Channel, "Angels of the Forest"
2008	Featured on Mongabay.com
2008	Featured in Plenty Magazine, February, "More than the Science"
2007	Featured on BBC radio on climate change and lemur reproduction
2007	Featured in "Wild Nature", produced by Rhett Butler
2006	Featured in Smithsonian Magazine, April Cover Article "For the Love of Lemurs"
2006	Featured in Award-winning National Public Radio show "Life on Earth, Madagascar Biodiversity", produced by Dan Grossman.
2006	Explorers Club Lecture Series, New York, NY
2006	Featured in BBC documentary special "Looking for Aye-Ayes with Miranda Stevenson"
2005	Featured in Natural History Magazine June Cover Article "Dance of the Sexes"
2004	Featured in Martin Kratt's National Geographic "Be the Creature" television series
2003	Royal Geographical Society Invited Speaker, London, UK
2002	Featured in David Attenborough's "Life of Mammals" documentary film
2001	Featured in the television documentary "Extinction", produced by Dan Mogulof, New York Times/Science Times and National Geographic Society
2000	Featured in "The Golden Bamboo Lemur", one-hour television special, NHK Japan
2000	Featured in Emmy Award winning documentary film "Me and Isaac Newton"
2000	Featured in Nova Adventure "On-Line Scientist, Madagascar", produced by Peter Tyson
2000	Profiled in book "The Eighth Continent" by Peter Tyson
1999	Featured in "Woman to Woman" Channel One Television, New York
1997	Featured in "To the Young Environmentalist: Lives Dedicated to Preserving the Natural World"
1996	Featured in "The Song of the Dodo: Island Biogeography in an Age of Extinctions", D. Quammen
1993	Featured in "For the Wild Places: Profiles in Conservation", Janet Bohlen

SCIENTIFIC PUBLICATIONS (SELECTED)

200+ publications in journals and books (details available upon request)

- Jones, J, Ratsimbazafy HJ, **Wright PC**, 2019 Last chance for Madagascar's biodiversity. Nature.
- Herrera, JP, Borgerson, C, Tongasoa, L, Andriamahazoarivosoa, P, Rasolofoniaina, BJR, Rakotondrafarasata, ER, Randrianasolo, JLRR, Johnson, SE, **Wright, PC**, Golden, CD. 2018. Estimating the population size of lemurs based on their mutualistic food trees. Journal of Biogeography.
- Dunham, AE, Razafindratsima OH, Rakotonirina P, and **Wright, PC**. 2018. Fruiting phenology is linked to rainfall variability in a tropical rainforest. Biotropica.
- Kling KJ & **Wright PC**. 2018. Conservation, Primates in The International Encyclopedia of Biological Anthropology, Wenda Trevathan (ed). John Wiley and Sons, Inc.
- Giordano RC, Rist CL, Parsons MB, Ramananjato R, **Wright PC**, Bliska JB, Bonds M, Gillespie TR. 2018. Behavioral and socio-economic risk factors of pathogenic enterobacteria infection and antibiotic resistance in Ranomafana commune, Madagascar. Emerging Infectious Diseases.
- Ragazzo, L. J., Zohdy, S., Velonabison, M., Herrera, J., **Wright, P. C.**, & Gillespie, T. R. (2018). *Entamoeba histolytica* infection in wild lemurs associated with proximity to humans. Veterinary parasitology, 249, 98-101.
- Zohdy S, Bisanzio D, Tecot S, **Wright PC**, Jernvall J. 2017. Aggression and hormones are associated with heterogeneity in parasitism and parasite dynamics in the brown mouse lemur. Animal Behaviour 132:109-19.
- Razafindratsima OH, Brown KA, Carvalho F, Johnson SE, **Wright PC**, Dunham AE. 2017. Edge effects on components of diversity and above - ground biomass in a tropical rainforest. Journal of Applied Ecology.
- Kappeler PM, Cuozzo FP, Fichtel C, Ganzhorn JU, Gursky-Doyen S, Irwin MT, Ichino S, Lawler R, Nekaris KAI, Ramanamanjato JB, Radespiel U, Sauther ML, **Wright PC**, Zimmermann E. 2017. Long-term field studies of lemurs, lorises, and tarsiers. Journal of Mammalogy 98 (3). 661-669.
- Farris ZJ, Gerber BD, Valenta K, Rafaliarison R, Razafimahaimodison JC, Larney E, Rajaonarivelo T, Randriana Z, **Wright PC**, Chapman CA. 2017. Threats to a rainforest carnivore community: A multi-year assessment of occupancy and co-occurrence in Madagascar. Biological Conservation. 210: 116-124.
- Ganzhorn JU, Arrigo - Nelson SJ, Carrai V, Chalise MK, Donati G, Droescher I, Eppley TM, Irwin MT, Koch F, Koenig A, Kowalewski MM, Mowry CB, Patel ER, Pichon C, Ralison J, Reisdorff C, Simmen B, Stalenberg E, Starrs D, Terboven J, **Wright PC**, Foley WJ. 2017. The importance of protein in leaf selection of folivorous primates. American Journal of Primatology 79(4):1-3.
- Donahue, M, Absanga A, Stumpf R, Weinrock D. and **Wright P.C**. 2019. Habitat disturbance and food species diversity drive extensive variability in the microbiome of a highly-specialized and critically endangered lemur species. Biology Letters.
- Hansford J., **Wright PC**, Rasoamiaramanana A., Pérez V.R., Godfrey L.R., Errickson, D.E., Thompson, T.,Turvey, S.T. (2018) Early Holocene human presence in Madagascar evidenced by exploitation of avian megafauna. Science Advances, 4, 6925.
- Zohdy S, Derfus K, Headrick EG, Andrianjafy MT, **Wright PC**, Gillespie TR. 2016. Small-scale land-use variability affects Anopheles spp. distribution and concomitant Plasmodium infection in humans and mosquito vectors in southeastern Madagascar. Malaria journal, 15:1.
- Eronen, J. T., Zohdy, S., Evans, A. R., Tecot, S. R., **Wright, P. C.**, & Jernvall, J. (2017). Feeding Ecology and Morphology Make a Bamboo Specialist Vulnerable to Climate Change. Current Biology, 27(21), 3384-3389.
- Scheffers, B. R., Edwards, D. P., Macdonald, S. L., Senior, R. A., Andriamahohatra, L. R., Roslan, N., Rogers, A.M., Haugaasen, T., **Wright, P.C.** & Williams, S. E. (2017). Extreme thermal heterogeneity in structurally complex tropical rain forests. Biotropica, 49(1), 35-44.
- Schwitzer C, Mittermeier RA, Johnson SE, Donati G, Irwin M, Peacock H, Ratsimbazafy J, Razafindramanana J, Louis Jr. EE, Chikhi L, Colquhoun IC, Tinsman J, Dolch R, LaFleur M, Nash S, Patel E, Randrianambinina B, Rasolofoharivelo T, **Wright PC**. 2014. Averting lemur extinctions amidst Madagascar's political crisis. Science 343: 842.
- Baden AL, **Wright PC**, Louis Jr EE, Bradley BJ. 2013. Communal nesting, kinship, and maternal success in a social primate. Behavioral Ecology and Sociobiology. 1939-50.
- Brown KA, Johnson SE, Parks KE, Holmes SM, Ivoandry T, Abram NK, Delmore KE, Ludovic R, Andriamaharoa HE, Wyman TM, **Wright PC**. 2013. Use of provisioning ecosystem services drives loss of functional traits across land use intensification gradients in tropical forests in Madagascar. Biological Conservation 161:118-127.
- **Wright PC**, et al., 2012. Long-term lemur research at Centre Valbio, Ranomafana National Park, Madagascar. In: Kappeler PM, and Watts DP, eds. Long-term field studies of primates. Springer p67-100.
- Gerber BD, Arrigo-Nelson SA, Karpanty SM, Kotschwar M, **Wright PC**. 2012. Spatial ecology of the endangered Milne-Edwards' sifaka (*Propithecus edwardsi*): do logging and season affect home range and daily ranging patterns? Int, J. of Primatology 33:305-321.
- **Wright, PC**. (1999). Lemur traits and Madagascar ecology: Coping with an island environment. Yearbook of Physical Anthropology 1999 42: 31-72.

BOOKS

- "Tarsiers: Past, Present and Future", Rutgers University Press
- "Madagascar and the Comoros", Lonely Planet Press
- "Madagascar: Forest of our Ancestors", Renoit Press
- "High Moon Over the Amazon: My Quest to Understand the Monkeys of the Night", Lantern Press, NY, NY
- "For the Love of Lemurs: My Life in the Wilds of Madagascar", Lantern Press, NY, NY

Further details available upon request.

Management Authority, FWHQ <managementauthority@fws.gov>

[EXTERNAL] Possible to amend permit application?

4 messages

Elise Lauterbur <lauterbur@gmail.com> Wed, Dec 11, 2019 at 11:35 AM
To: "Management Authority, FWHQ" <managementauthority@fws.gov>

Good morning,

Your office is currently processing our permit application **US56547D/9, and I have just been notified that we would like to import some additional samples from the same individual animals. (Eg. Currently the permit requests blood samples for three species, we would also like to import skin biopsy samples from the same individuals from which we will obtain the blood samples.)

Could you please tell me if it is possible to amend this permit application, or should we prepare a completely new application for these additional samples?

Thank you,
Elise

Management Authority, FWHQ <managementauthority@fws.gov> Thu, Dec 19, 2019 at 9:37 AM
To: Elise Lauterbur <lauterbur@gmail.com>

Dear Eilse,

Thank you for your inquiry. You may submit additional information to this email to be added to your application file since your application is still being reviewed.

We appreciate your time and consideration.

****

U.S. Fish and Wildlife Service
International Affairs Program
Division of Management Authority
Branch of Permits
1-800-358-2104
Reply to: ManagementAuthority@fws.gov, http://www.fws.gov/international/permits/
Sign up for our e-newsletter to learn how we're working around the globe to protect species and their habitats!
Going abroad? Be Informed. Buy Informed.
****

Mailing address:
ATTN DIVISION OF MANAGEMENT AUTHORITY - BRANCH OF PERMITS
U.S. FISH & WILDLIFE SERVICE HEADQUARTERS
5275 LEESBURG PIKE, MS: IA
FALLS CHURCH, VA 22041-3803

[Quoted text hidden]

Elise Lauterbur <lauterbur@gmail.com> Wed, Jan 8, 2020 at 1:09 PM
To: "Management Authority, FWHQ" <managementauthority@fws.gov>

Dear FWS,

Thank you very much. I have attached additional information to this email in two formats - The first file, "CITES_import_application_2019_12_27.doc" is an updated version of the application as it was originally submitted, with the additional information included alongside the original information. To facilitate seeing the additional information, I have also attached "CITES_import_application_2019_12_27_additional.doc" which includes only the additional information, in the appropriate sections.

Thank you again for allowing us to add this additional information while the application is being reviewed.
Best,
Elise
[Quoted text hidden]

2 attachments

CITES_import_application_2019-12-27_additional.doc
38K

CITES_import_application_2019-12-27.doc
247K

Elise Lauterbur <lauterbur@gmail.com> Wed, Jan 8, 2020 at 1:11 PM
To: "Management Authority, FWHQ" <managementauthority@fws.gov>

My apologies, also attached here is the CV of the additional collaborator.

Thanks,
Elise
[Quoted text hidden]

Ashish Toshniwal CV.pdf
198K

13. Jan. 2020

CITES import permit application
Institute for the Conservation of Tropical Environments

5. For EACH animal/specimen involved in the proposed activity provide:

a. Scientific name	**b. Common name**	**c. Birth/Hatch Date**	**d. Wild or captive-born**	**e. Quantity**	**f. Gender**	**g. Permanent markings**	**h. Type of sample or product**	**9.b. Date sampled**
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Skin Biopsy	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Skin Biopsy	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Skin Biopsy	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Skin Biopsy	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Skin Biopsy	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Skin Biopsy	Planned January 2020
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	*Prolemur simus*	Skin Biopsy	Planned January 2020
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	*Prolemur simus*	Skin Biopsy	Planned January 2020
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	*Prolemur simus*	Skin Biopsy	Planned January 2020
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	*Prolemur simus*	Skin Biopsy	Planned January 2020
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	*Prolemur simus*	Skin Biopsy	Planned January 2020

Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	*Prolemur simus*	Skin Biopsy	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Skin Biopsy	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Skin Biopsy	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Skin Biopsy	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Skin Biopsy	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Skin Biopsy	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Skin Biopsy	Planned January 2020

JUSTIFICATION FOR REQUESTED ACTIVITY.

10. Provide a detailed statement justifying the proposed activity, particularly the following:

a. Describe the purpose of your proposed activity.

In addition to the previously-mentioned goals, the white blood cells and skin biopsy taken from *P. simus* and *H. aureus* individuals will be used to generate immortalized cell lines. These cells will be used for molecular and metabolic investigation of the incredible adaptation that these animals have made to the ingestion of lethal amounts of cyanide. For these analyses, many state-of-the-art molecular biology and metabolic experiments will be performed. Though a few reports state that the cyanide adaptation is a physiological effect, there is ample evidence indicating that most tissues and organs in the body can be exposed to high doses of cyanide during the normal behavior of animals. Understanding the basic biology behind this trait will be extremely helpful to understand the habitat and diet of these animals. It could also enable us to better design therapies for cyanide toxicity in humans and other animals, which is a significant risk.

b. Description of the technical expertise of each person as it relates to the proposed activities.

Dr. Ashish Toshniwal is a Postdoctoral Fellow in the Biochemistry Department at the University of Utah, where he is studying cyanide as a metabolic toxin and developing novel therapies for cyanide toxicity. With his expertise in molecular biology and metabolism, he is investigating the molecular biology and metabolic underpinnings of adaptation in *Hapalemur aureus* and *Prolemur simus*. Please see CV attached.

11. A statement on how the activities will **enhance or benefit the wild population**:

In addition to the previously-described benefits, the skin biopsies will enhance our understanding of the unique nutritional needs of bamboo lemurs. Using the white blood cells and fibroblasts isolated from the skin biopsy, we will study the molecular and metabolic basis of adaptation to high amount of cyanide consumption in *P. simus* and *H. aureus*. This can help us to better understand how the cyanide tolerance trait was developed during evolution. In addition to that, these studies will also decode why the *P. simus* species specifically depend on this specialized diet of bamboo wood with a high cyanide content. This might give us novel insights into how cyanide-consuming lemurs and the bamboo plants have coevolved. Moreover, the results of this study can lead us to design novel conservation strategies for these endangered species of lemurs.

13. Jan. 2020

CITES import permit application
Institute for the Conservation of Tropical Environments

5. For **EACH** animal/specimen involved in the proposed activity provide:

a. Scientific name	**b. Common name**	**c. Birth/Hatch Date**	**d. Wild or captive-born**	**e. Quantity**	**f. Gender**	**g. Permanent markings**	**h. Type of sample or product**	**9.b. Date sampled**
Prolemur simus	Greater Bamboo Lemur	Estimated 2018	Wild	4	Male	Microchip #0A01750051	Blood (2mL tubes)	Aug. 1 2019
Prolemur simus	Greater Bamboo Lemur	Estimated 2018	Wild	1	Male	Microchip #0A01750051	Buffy Coat (2mL tube)	Aug. 1 2019
Prolemur simus	Greater Bamboo Lemur	Estimated 2018	Wild	1	Male	Microchip #0A01750051	Hair (plucked, stored in 2mL tube)	Aug. 1 2019
Prolemur simus	Greater Bamboo Lemur	Estimated 2018	Wild	1	Male	Microchip #0A01750051	Plasma (2mL tube)	Aug. 1 2019
Prolemur simus	Greater Bamboo Lemur	Estimated 2018	Wild	2	Male	Microchip #0A01750051	Protein Saver Card	Aug. 1 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	4	Female	Microchip #0A02011231	Blood (2mL tubes)	Aug. 1 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	Microchip #0A02011231	Buffy Coat (2mL tube)	Aug. 1 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	Microchip #0A02011231	Hair (plucked, stored in 2mL tube)	Aug. 1 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	Microchip #0A02011231	Plasma (2mL tube)	Aug. 1 2019
Prolemur	Greater	Unknown	Wild	3	Female	Microchip	Protein	Aug. 1

simus	Bamboo Lemur	(adult)				#0A02011231	Saver Card	2019
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	3	Male	Microchip #0A01750201	Blood (2mL tubes)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	1	Male	Microchip #0A01750201	Buffy Coat (2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	1	Male	Microchip #0A01750201	Hair (plucked, stored in 2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	1	Male	Microchip #0A01750201	Plasma (2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	2	Male	Microchip #0A01750201	Protein Saver Card	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	3	Female	Microchip #0A01752151	Blood (2mL tubes)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	Microchip #0A01752151	Buffy Coat (2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	Microchip #0A01752151	Hair (plucked, stored in 2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	Microchip #0A01752151	Plasma (2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	2	Female	Microchip #0A01752151	Protein Saver Card	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	3	Female	Microchip #0A01750829	Blood (2mL tubes)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	1	Female	Microchip #0A01750829	Buffy Coat (2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	1	Female	Microchip #0A01750829	Hair (plucked, stored in	Aug. 2 2019

							2mL tube)	
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	1	Female	Microchip #0A01750829	Plasma (2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	2	Female	Microchip #0A01750829	Protein Saver Card	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	3	Female	Microchip #0A01752351	Blood (2mL tubes)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	*Prolemur simus*	Buffy Coat (2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	*Prolemur simus*	Hair (plucked, stored in 2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	*Prolemur simus*	Plasma (2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	*Prolemur simus*	Protein Saver Card	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	2	Female	*Prolemur simus*	Blood (2mL tubes)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	3	Male	Microchip #0A01751807	Blood (2mL tubes)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	1	Male	Microchip #0A01751807	Buffy Coat (2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	1	Male	Microchip #0A01751807	Hair (plucked, stored in 2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	1	Male	Microchip #0A01751807	Plasma (2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (subadult)	Wild	2	Male	Microchip #0A01751807	Protein Saver Card	Aug. 2 2019

Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	3	Male	Microchip #0A01750603	Blood (2mL tubes)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Male	Microchip #0A01750603	Buffy Coat (2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Male	Microchip #0A01750603	Hair (plucked, stored in 2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Male	Microchip #0A01750603	Plasma (2mL tube)	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	2	Male	Microchip #0A01750603	Protein Saver Card	Aug. 2 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	5	Female	Microchip #0A01751302	Blood (2mL tubes)	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	Microchip #0A01751302	Buffy Coat (2mL tube)	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	Microchip #0A01751302	Hair (plucked, stored in 2mL tube)	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	Microchip #0A01751302	Plasma (2mL tube)	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	3	Female	Microchip #0A01751302	Protein Saver Card	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	4	Female	Microchip #0A01750920	Blood (2mL tubes)	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	Microchip #0A01750920	Buffy Coat (2mL tube)	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Female	Microchip #0A01750920	Hair (plucked, stored in 2mL tube)	Aug. 3 2019
Prolemur	Greater	Unknown	Wild	1	Female	Microchip	Plasma	Aug. 3

simus	Bamboo Lemur	(adult)				#0A01750920	(2mL tube)	2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	2	Female	Microchip #0A01750920	Protein Saver Card	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	4	Male	Microchip #0A01751109	Blood (2mL tubes)	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Male	Microchip #0A01751109	Buffy Coat (2mL tube)	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Male	Microchip #0A01751109	Hair (plucked, stored in 2mL tube)	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Male	Microchip #0A01751109	Plasma (2mL tube)	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	2	Male	Microchip #0A01751109	Protein Saver Card	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Male	Microchip #0A01752527	Blood (2mL tubes)	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	4	Male	Microchip #0A01752527	Buffy Coat (2mL tube)	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Male	Microchip #0A01752527	Hair (plucked, stored in 2mL tube)	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Male	Microchip #0A01752527	Plasma (2mL tube)	Aug. 3 2019
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	2	Male	Microchip #0A01752527	Protein Saver Card	Aug. 3 2019
Hapalemur aureus	Golden Bamboo Lemur	November 2016 (infant)	Wild	2	Female	None, preserved in freezer at Centre ValBio Madagascar	Tissue (2mL tube)	Found dead by local research technician,

								January 2016
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood in buffer (9mL tube: 3mL blood, 6mL buffer)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood in buffer (9mL tube: 3mL blood, 6mL buffer)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood in buffer (9mL tube: 3mL blood, 6mL buffer)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood in buffer (9mL tube: 3mL blood, 6mL buffer)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood in buffer (9mL tube: 3mL blood, 6mL buffer)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood in buffer (9mL tube: 3mL blood, 6mL buffer)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood in buffer (9mL tube: 3mL blood, 6mL buffer)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood in buffer (9mL tube: 3mL blood, 6mL buffer)	Planned January 2020
Hapalemur aureus	Golden Bamboo	Unknown (adult)	Wild	1	Unknown	Unknown	Blood in buffer (9mL	Planned January

	Lemur						tube: 3mL blood, 6mL buffer)	2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood in buffer (9mL tube: 3mL blood, 6mL buffer)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood in buffer (9mL tube: 3mL blood, 6mL buffer)	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood in buffer (9mL tube: 3mL blood, 6mL buffer)	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood in buffer (9mL tube: 3mL blood, 6mL buffer)	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood in buffer (9mL tube: 3mL blood, 6mL buffer)	Planned January 2020
Eulemur	Red-	Unknown	Wild	1	Unknow	Unknown	Blood in	Planned

rufifrons	fronted brown lemur	(adult)			n		buffer (9mL tube: 3mL blood, 6mL buffer)	January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Hapalemu	Golden	Unknown	Wild	1	Unknow	Unknown	Skin Biopsy	Planned

r aureus	Bamboo Lemur	(adult)			n			January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Skin Biopsy	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Skin Biopsy	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Skin Biopsy	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Skin Biopsy	Planned January 2020
Hapalemur aureus	Golden Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Skin Biopsy	Planned January 2020
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	*Prolemur simus*	Blood/plasma (3mL tube)	Planned January 2020
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	*Prolemur simus*	Blood/plasma (3mL tube)	Planned January 2020
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	*Prolemur simus*	Blood/plasma (3mL tube)	Planned January 2020
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	*Prolemur simus*	Blood/plasma (3mL tube)	Planned January 2020
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	*Prolemur simus*	Blood/plasma (3mL tube)	Planned January 2020
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	*Prolemur simus*	Blood/plasma (3mL tube)	Planned January 2020
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	*Prolemur simus*	Skin Biopsy	Planned January 2020
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	*Prolemur simus*	Skin Biopsy	Planned January 2020
Prolemur	Greater	Unknown	Wild	1	Unknow	*Prolemur*	Skin Biopsy	Planned

simus	Bamboo Lemur	(adult)			n	*simus*		January 2020
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	*Prolemur simus*	Skin Biopsy	Planned January 2020
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	*Prolemur simus*	Skin Biopsy	Planned January 2020
Prolemur simus	Greater Bamboo Lemur	Unknown (adult)	Wild	1	Unknown	*Prolemur simus*	Skin Biopsy	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Blood/plasma (3mL tube)	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Skin Biopsy	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Skin Biopsy	Planned January 2020

Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Skin Biopsy	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Skin Biopsy	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Skin Biopsy	Planned January 2020
Eulemur rufifrons	Red-fronted brown lemur	Unknown (adult)	Wild	1	Unknown	Unknown	Skin Biopsy	Planned January 2020

6. The current location of the specimen(s):
Name: Dr. Patricia C. Wright
Address: 33 BP
City: Ranomafana
State/Province: Ifanadiana
Postal Code: 312
Country: Madagascar

7. Recipient:
Name: Dr. Patricia C. Wright
Address: N-203 Social and Behavioral Sciences Building, Stony Brook University
City: Stony Brook
State: New York
County, Postal Code: Suffolk, 11794-4310

SOURCE OF SPECIMEN

8. N/A

9. For **EACH** animal/specimen **taken from the wild**, provide the following:

a. Scientific name and common name: *Prolemur simus*, Greater Bamboo Lemur
Hapalemur aureus, Golden Bamboo Lemur
Eulemur rufifrons, Red-fronted Brown Lemur

b. Specific location of where, when, and by whom (name and address) the specimen was removed from the wild:
Species: *Prolemur simus*
Where: Karianga, Ivato, District Vondrozo, Madagascar

When: Aug. 1 – 3 2019. See table above for exact dates. Second sampling planned for January 2020 during translocation.
By whom: Dr. Patricia C. Wright, N-203 Social and Behavioral Sciences Building, Stony Brook University, Stony Brook NY 11794-4310, USA

Species: *Hapalemur aureus* and *Eulemur rufifrons*
Where: Ranomafana National Park, Districts Haute Matsiatra and Vatovavy-Fitovinany, Madagascar
When: January 2020. Collecting has not yet taken place.
By whom: Morgan Chaney, 750 Hilltop Drive, Room 226, Ken State University, Kent, Ohio 44242

c. Purpose of removal and length of time held in captivity:

Prolemur simus:

Purpose: Animals were captured to fit them with radio collars and be examined by a veterinarian prior to a translocation from a disturbed region to a protected region, Ranomafana National Park. They will be re-captured in January of 2020 for the translocation. Human disturbance in their current range negatively affects the lemurs' food supply and has in the past included illegal poaching. In their new range they will be both protected from human disturbance and have the potential to contribute to the genetic diversity of the severely declining population in the protected region. The radio collars will provide the means to track the animals when they are release in the National Park. While they were anesthetized, blood samples and measurements were also taken for the analyses described below, including understanding the population genetics of the species.

Length of time: Each animal was held for approximately 5-8 hours between time of darting and time of release. They were released after full recovery from the effects of the anesthesia.

Hapalemur aureus and Eulemur rufifrons:

Purpose: Animals are being darted and captured for the purpose of obtaining blood samples for the research described below in question 10 and in detail in the attachment "DDRIG Proposal_Chaney.docx" The skin biopsy will be taken for isolating fibroblasts. In short, the blood samples will be used to understand the genetics and biochemistry of cyanide adaptation at physiological levels. Both blood cells and skin cells will be used to examine whether cultured cells from these animals exhibit unusual tolerance to a high amount of cyanide. This species eats 12-50 times the lethal dose for a similar weight animal.

Length of time: Each animal will be held for approximately 3-5 hours between time of darting and time of release. They will be released after full recovery from the effects of the anesthesia.

d. If and how any remuneration, either financial or in-kind, was provided for taking or capturing animals or for the collection of samples.

The supervising veterinarian, Dr. Hajanirina Rakotondrainibe, was and will be paid a standard rate for his services in evaluating and monitoring the health of the lemurs. Darting technician Velotsara Jean Baptiste was and will be paid a standard rate for his services in darting the animals.

No remuneration of any kind was or will be provided for the samples themselves.

e. Your efforts to use captive specimens, or parts thereof, in lieu of taking animals from the wild:

The *Prolemur simus* samples are from animals that are part of a translocation program to transfer animals from a disturbed region to a protected region, to protect these individuals and the future genetic diversity of the species. Currently, the only population in a National Park consists of two related individuals, a father and daughter. Thus, to understand the genetic consequences of this translocation, the samples must come from the individuals translocated, and their capture for fitting radio collars and health examinations provided the opportunity to collect high quality DNA samples without additional disturbance. High quality samples, such as from blood, are imperative because additional conservation genetic studies will be carried out including analyses of nutritional and disease adaptations in this population.

The *Hapalemur aureus* samples are from wild animals eating their natural diet. There are no *H. aureus* currently living in captivity anywhere in the world. Thus, to understand anything about their population genetics and adaptation, including inbreeding, population connectivity, and nutritional adaptation, requires sampling wild individuals in their native range. The *Eulemur rufifrons* samples will be used to compare the nutritional adaptations of a non-bamboo eating species (*E. rufifrons*) with the bamboo specialized and cyanide adapted *H. aureus*. It is necessary that samples are taken from individuals that live in the same habitat and are exposed to the same dietary options as the *H. aureus* individuals sampled, thus we cannot use samples from captive *Eulemur.*

f. Copies of your foreign or domestic collecting permit.

See attached **Autorisation de Recherche (Research Authorization)** from the Gestion des Ressources Naturelles Renouvelables et des Ecosystemes (Department for Management of Renewable Natural Resources and Ecosystems) in Madagascar. Original in French, translation provided. This permit applies to the *Prolemur simus* samples obtained in August 2019 listed in question 5.

A permit has not yet been granted for the additional *P. simus* samples that will be obtained during the second darting procedure immediately prior to translocation. Currently, the Malagasy government typically grants permits only once the research team is in Madagascar and ready to proceed with the project. In this case that will be January 2020. A permit application has not yet been submitted to the Gestion des Ressources Naturelles Renouvelables et des Ecosystemes for the *Hapalemur aureus* sampling project. Based on our previous successes obtaining permits for similar studies (including the included *P. simus* study) we anticipate receiving authorization for both of these sampling protocols. However if a permit is not granted, the *H. aureus* project will not go forward and no samples will be collected. If the additional *P. simus* sampling is not approved, they will be transported (as previously approved in the Autorisation de Recherche) but no additional samples taken.

g. Documentation showing that the specimen(s) were legally obtained by the applicant.

See attached **Proces-Verbal de Constatation (Report of Observation)** from the Chef Cantonnement de l'Environment de l'Écologie et des Forêts (Station head for the Environment, Ecology, and Forests Department) in Vondrozo, Madagascar. Original in French, translation provided. This documentation is for the samples obtained during the first *Prolemur simus* darting, prior to translocation. In Madagascar, the Proces-Verbal de Constation is only completed upon completion of sampling, thus we do not yet have it for the second round of *P. simus* sampling (during the transportation part of the translocation project) or the *H. aureus* sampling. As this document is required to obtain a CITES export permit from Madagascar, neither the additional *P. simus* samples nor the *H. aureus* samples will be exported from Madagascar, and thus not imported to the United States, if it is not granted.

h. Copies of any applicable State, Tribal, Federal, or Foreign government permits or licenses that authorized the removal of this animal from the wild.

See attached **Research Authorization**, described above.

JUSTIFICATION FOR REQUESTED ACTIVITY.

10. Provide a detailed statement justifying the proposed activity, particularly the following:

a. Describe the purpose of your proposed activity.

Of a total world population estimated around 500-600 individuals, there are only two wild *Prolemur simus* individuals within a protected area. The rest are in fragmented populations subject to varying amounts of human disturbance. The two individuals living in a protected area, Ranomafana National Park (RNP) are a father and daughter, and despite the daughter having reached breeding age at least 5 years ago, they have not reproduced with each other nor have other *P. simus* individuals immigrated to expand the group. Thus the goal of the translocation project during which these samples were collected is twofold:

1. Increase the population size within RNP, hopefully increasing breeding opportunities and thus genetic diversity in a protected population;
2. Move a family group of *P. simus* individuals from a disturbed habitat in which they are currently at risk to the protected National Park.

The translocation occurs in two stages. First, the family group to be translocated has been identified, radio collared and examined, and the relevant samples taken. They were released at the darting site for monitoring until the translocation itself, when they will be re-captured and transported to Ranomafana National Park. Additional samples will be taken at this time.

The objectives are:

1. Translocate a family group from the unprotected region of Karianga to RNP.
    1. Fit captured individuals with radio collars to facilitate tracking once released,
    2. Examine the health and parasite load of the individuals to be translocated, both pre- and post-translocation,
    3. Monitor their health and behavior after capture in their native range,
    4. Recapture and translocate to RNP

2. While anesthetized for the above procedures (#1/2 and #4), obtain blood (including buffy coat, plasma, and protein) and hair samples for further health, nutrition adaptation, and disease adaptation analyses.

The objectives of the *Hapalemur aureus* project are twofold:

1. Bamboo lemurs, including *H. aureus*, have some of the most specialized diets among all primates, and possibly the only primates genetically adapted to cope with high dietary cyanide (12-50 times the expected lethal dose). Blood samples from *H. aureus* and a related species with overlapping range, *Eulemur fulvus,* will be used to improve our understanding of their adaptation to this lethal toxin.
2. *H. aureus* is considered to be Critically Endangered by the IUCN, but currently nothing is known about their population genetics. These samples will also be used to determine the genetic health of the species, including testing for inbreeding, current and historical population size, and population fragmentation.

Four different types of analyses will be performed with blood samples:

1. Population demographic analyses to investigate the population history, heterozygosity, and potential inbreeding of both *P. simus* and *H. aureus*. Understanding these aspects of the population are important for continued conservation efforts. Population genetic analysis of *P. simus* has only previously been done using only five individuals from across the species' current range. This analysis will more than double the sample set, and allow greater depth of understanding of the population and inbreeding risk. Population genetic analysis of *H. aureus* has never been done.

2. Genomic adaptation analyses to investigate ecological correlates of disease adaptation in both *P. simus* and *H. aureus*. Wild lemurs are susceptible to many diseases, and this investigation will compare disease adaptations in populations that have been isolated for many generations, with different amounts of human contact (thus increased exposure to human and livestock diseases). In particular, both *P. simus* and *H. aureus* may have less exposure to vector-borne diseases than other lemur species, since their high cyanide diet may deter ectoparasites that spread disease. This analysis has not previously been carried out, and makes use of novel bioinformatic methods that require high quality assembled genomes with maximum contiguity of the genomic scaffolds. To this end, we will be conducting third-generation (long-read) whole genome sequencing, which requires high molecular weight DNA.

3. RNA expression analyses to investigate protein expression patterns associated with cyanide adaptation, which is unique among primates to bamboo lemurs. While previous work has identified genes involved with *P. simus* and *H. aureus* cyanide adaptation, the protein coding changes found are insufficient to entirely explain the extent of this unique adaptation. RNA expression analyses require blood samples, since the detoxification enzymes and cellular respiration associated proteins are expected to be expressed in the blood to combat circulating cyanide after it is ingested by the lemurs. (Cyanide production is naturally used by the bamboo they eat as an anti-herbivory strategy.)

Because the translocation protocol involves capturing the animals twice (once for health assessment and radio collaring to facilitate monitoring before transportation, and once for the transportation itself), we will compare RNA expression in *P. simus* at two times of the year, once during the dry season when the animals are eating very little cyanide, and once during the wet season when the animals are eating a great deal of cyanide (the bamboo produces cyanide mainly in its actively growing structures). This will allow a seasonal comparison to validate that expression patterns are associated with dietary cyanide.

4. Chemical composition analyses of the blood to investigate concentrations of free cyanide and its primary metabolite, thiocyanate in both *P. simus* and *H. aureus*. These are expected in the blood based on previous non-invasive studies using urine, but their concentrations have never been directly measured. This will complement the previously-described RNA expression analyses, and requires blood samples. Likewise, we will compare chemical composition in *P. simus* at two times of the year.

5. In addition to these above-mentioned goals, the white blood cells and skin biopsy taken from *P. simus* and *H. aureus* individuals will be used to generate immortalized cell lines. These cells will be used for molecular and metabolic investigation of the incredible adaptation that these animals have made to the ingestion of lethal amounts of cyanide. For these analyses, many state-of-the-art molecular biology and metabolic experiments will be performed. Though a few reports state that the cyanide adaptation is a physiological effect, there is ample evidence indicating that most tissues and organs in the body can be exposed to high doses of cyanide during the normal behavior of animals. Understanding the basic biology behind this trait will be extremely helpful to understand the habitat and diet of these animals. It could also enable us to better design therapies for cyanide toxicity in humans and other animals, which is a significant risk.

The darting of lemurs is strictly regulated by Madagascar's Ministry of the Environment. Proposals for permits need to be submitted and approved before darting takes place. Centre ValBio's Institute for the Conservation of Tropical Environments ICTE representative in Madagascar's capital, Dr. Benjamin Andriamihaja, facilitates the submission of Dr. Patricia C. Wright's permit proposals. In order to dart animals, researchers must submit a proposal to Madagascar National Parks (MNP) describing why the darting is needed. The proposal for darting and translocation of *P. simus* was approved (please see attached Autorisation de Recherche and translation). The proposal for darting of *H. aureus* has not yet been submitted, but is expected to be approved. If it is not approved, the darting will not be carried out and no samples will be taken.

The process of darting will follow a standard methodology. Darting is performed by trained specialists, including Centre ValBio's affiliated veterinarian Dr. Hajanirina RAKOTONDRAINIBE (please see attached CVs). The darting team uses a CO2 air rifle, which launches light-weight 9-mm darts. The darts inject Telazol® at 10 mg/kg of body weight intramuscularly. Lemurs are caught individually in a large net held by four people as they fall.

Each anesthesized lemur is checked by the veterinarian (heart rate, lung listened to, body temperature taken), weighed and measured. Ectoparasites are removed for further investigation, the animals are checked for scars, nipples on the females are checked for milk, measurement of male testes is collected and other protocol specific measurements are taken. Blood is drawn and hair samples plucked. The data on each individual lemur is kept in a database at Centre ValBio. Each animal will receive a radio collar so that he/she can be followed. The ATM radio collars have been used previously on this species successfully. After the medical check-up and blood and hair sampling, the animals are allowed to recuperate from the Telazol in light-weight sacks (recuperation time ~ 3 hours) before they are released back to the darting site. Animals are followed and monitored for two to five hours to ensure complete recovery.

For further methodological details of darting, translocation, and sampling, please see the attached research proposals: "Updated_proposal_translocation_July19.docx" and its translation, and "DDRIG Proposal_Chaney.docx". Its important to note that, number of animals of *H. aureus and E. rufifrons* being tranquilized for taking blood will not change, as proposed previously. Skin biopsy will be taken from same individuals which are tranquilized. Moreover, many *P. simus* will be tranquilized for translocation purpose, which is already approved.

b. Description of the technical expertise of each person as it relates to the proposed activities.

Dr. Patricia Wright, director of the Institute for Conservation of Tropical Environments, has been researching and protecting lemurs in Madagascar for 30 years, including helping to establish Ranomafana National Park to protect *Prolemur simus* and *Hapalemur aureus*. She has supervised more than two dozen lemur darting procedures in that time, including both of those species as well as other lemur species. Please see CV attached.

Dr. Hajanirina Rakotondrainibe is a wildlife veterinarian who has specialized in lemurs since 2007, and is a member of the Malagasy Order of Veterinary Doctors. He performed and will be performing the health monitoring and evaluation for all aspects of these projects. Please see CV attached.

Velotsara Jean Baptiste is a technician at Centre ValBio, Dr. Patricia Wright's research station, who specializes in lemur darting. He is the main darting technician at Centre ValBio and has experience safely darting *Prolemur simus*, *Hapalemur aureus,* and *Eulemur fulvus.*

Dr. Tobias Gräßle is a wildlife veterinarian who specializes in primate disease. He provided additional sampling supervision and health evaluation during the initial *Prolemur simus* capture. Please see CV attached.

Dr. Sina Feyer is a wildlife veterinarian who provided additional sampling supervision and health evaluation during the initial *Prolemur simus* capture. Please see CV attached.

Morgan Chaney is a PhD candidate in the Anthropology Department at Kent State University, where he is studying cyanide adaptation in *Hapalemur aureus* and *Prolemur simus*. Please see CV attached.

Dr. Ashish Toshniwal is a Postdoctoral Fellow in the Biochemistry Department at the University of Utah, where he is studying cyanide as a metabolic toxin and developing novel therapies for cyanide toxicity. With his expertise in molecular biology and metabolism, he is investigating the molecular biology and metabolic underpinnings of adaptation in *Hapalemur aureus* and *Prolemur simus*. Please see CV attached.

c. Copies of contracts, agreements or other documents that identify persons involved and dates of activities for which authorization is being requested.

Please see attached **Research Authorization**, described in 9f, that applies to samples obtained during the *Prolemur simus* translocation project. Please see attached "Chaney IACUC approved.pdf" that applies to samples that will be obtained for *Hapalemur aureus* nutrition and population genetics project.

11. A statement on how the activities will **enhance or benefit the wild population**:

Translocation:

The goal of the P. simus translocation project is to translocate a family group of P. simus from a threatened forest fragment to a protected national park where two individuals of the same species already reside. The translocation benefits the lemurs being translocated because they will go from a habitat where they are unwanted and threatened by agriculture and farmers (who have asked for their removal due to crop raiding) to pristine forest in a national park where they will be protected. This also benefits the two lemurs within the park because they are a non-breeding pair who have no hope for producing offspring without the introduction of new Prolemur simus individuals. The blending of two unrelated family groups benefits Prolemur simus as a whole by increasing genetic diversity and therefore survivability of the species. The associated sampling will benefit the wild population in two ways, described below.

Understanding the population genetics of *Prolemur simus* and *Hapalemur aureus*:

The collection of the *P. simus* samples is directly associated with the translocation project. The collection of both *P. simus* and *H. aureus* samples benefits their wild populations in three ways:

1. Understanding the population genetics of both species.
    a. *P. simus:* Population genetic analysis of *P. simus* has only previously been done using only five individuals from across the species' current range. This analysis will more than double the sample set, and allow greater depth of understanding of the population and inbreeding risk. Currently, levels of inbreeding are unknown in any of its populations. The historical population size of *P. simus* is estimated to be quite large, so current heterozygosity within the species may be higher than expected relative to its current population size. On the other hand, the current population is heavily fragmented, which may have resulted in each fragmented population being heavily inbred. This information about how inbred and how genetically fragmented the populations of this species are will impact future management decisions. The current translocation project is an urgently-needed stopgap, and the samples obtained during this process will improve future management plans.
    b. *H. aureus:* Population genetic analysis of *H. aureus* has never been done. As a result, nothing is known about their population genetics. Analyses of the

genetic health of the species, including testing for inbreeding, current and historical population size, and population fragmentation, are urgently needed. Understanding these aspects of the population are important for continued conservation efforts. This information will impact future management decisions, including whether or not translocations or breeding programs are necessary for the health of the species.

2. Understanding of the unique nutritional needs of both species. The natural diet of both *P. simus* and *H. aureus* are heavily (90+%) dependent on woody bamboo, including *Cathariostachys madagascariensis*. Many woody bamboos in Madagsacar, including *C. madagascariensis*, produce cyanide as an anti-herbivory mechanism, thus these two species are the only known cyanide adapted primates.
    a. It is hypothesized that woody bamboo, particularly *C. madagascariensis,* provides a healthier diet for *P. simus* and *H. aureus* than other foods they may find in anthropogenically disturbed areas, such as rice and cassava. Not only do *P. simus* and *H. aureus* rely on specific species of bamboo, they may rely on bamboo stands growing under conditions that result in specific nutrient profiles. However, it is currently unknown if cyanide is an important aspect of a normal diet for these two species. For example, a high cyanide diet may deter ectoparasites, thus protecting them from some disease (see point 3, below).
    b. By comparing the transcriptomes and blood chemistry of these two cyanide adapted species to those of a related species that shares their habitat (*Eulemur rufifrons*), both during a season of high cyanide ingestion and one of low cyanide ingestion, we can better understand how and why *P. simus* and *H. aureus* are so dependent on woody bamboo and, perhaps, cyanide. Because this reliance on woody bamboo may include stands growing under conditions that result in specific nutrient profiles, this research will improve current conservation efforts that include plans to propagate and re-establish stands of bamboo by improving our understanding of how and why their diets are so dependent on woody bambo.
    c. Using the white blood cells and fibroblasts isolated from the skin biopsy, we will study the molecular and metabolic basis of adaptation to high amount of cyanide consumption in *P. simus* and *H. aureus*. This can help us to better understand how the cyanide tolerance trait was developed during evolution. In addition to that, these studies will also decode why the *P. simus* species specifically depend on this specialized diet of bamboo wood with a high cyanide content. This might give us novel insights into how cyanide-consuming lemurs and the bamboo plants have coevolved. Moreover, the results of this study can lead us to design novel conservation strategies for these endangered species of lemurs.

3. Investigating ecological correlates of disease adaptation in both species. Wild lemurs are susceptible to many diseases. Some they are exposed to naturally, and some through increased exposure to humans and their livestock. In particular, both *P. simus* and *H. aureus* may have less exposure to vector-borne diseases than other lemur species, since their high cyanide diet may deter ectoparasites that spread disease. Understanding the extent of these adaptations, and thus their

susceptibility, can inform conservation decisions with respect to controlling disease transmission to these endangered species.

Neither the sampling itself, nor the purposes of the research, will be detrimental to the survival of this species, nor will it be used for any commercial purposes.

All information gathered from this research, and its conservation implications, will be made available in published documents and research articles. We intend to publish this research in scientific journals, as well as make it available to the Madagascar National Parks and conservation organizations.

12. If live specimens are to be held in captivity as part of the proposed activity:

N/A, animals are not being maintained in captivity and were released immediately upon recovery from anaesthesia.

IMPORTS, EXPORTS, OR RE-EXPORTS

13. For shipment of LIVE specimens…

N/A, no live specimens are to be transported.

14. For import of live southern white rhinocerouses…

N/A, no rhinocerouses are involved in this work.

15. For import of LIVE CITES Appendix-1 listed marine mammal species…

N/A, no marine mammals are involved in this work.

16. For import of CITES **Appendix-1 listed species,** provide information to show the import is not for primarily commercial purposes:

Please see attached **Autorisation de Recherche (Research Authorization)** from the Gestion des Ressources Naturelles Renouvelables et des Ecosystemes (Department for Management of Renewable Natural Resources and Ecosystems) in Madagascar. Original in French, translation provided.

This research authorization shows that the imported samples will be used for conservation research, animal health evaluation, and basic research purposes only.

17. For export…

N/A, no samples will be exported from the United States of America.

18. If the specimen is being re-exported…

N/A, no samples will be re-exported from the United States of America.

CURRICULUM VITAE

Dr. Ashish Toshniwal

PostDoctoral Fellow
C/O [redacted]
HHM
Professor of Biochemistry
University of Utah School of Medicine
15 N. Medical Drive East Room 5520A
Salt Lake City, UT 84112-5650
Phone: +1-321-444-5752
email: ashish.toshniwal@biochem.utah.edu
ashishgtoshniwal@gmail.com

Education and Research Experience

- **Postdoctoral Fellow** (Oct 2019- current) at Prof. Jarred Rutter's Laboratory at Department of Biochemistry, University of Utah, Salt Lake City, Utah. I am keen to understand how mitochondrial metabolism affects cell viability and function. The primary aim of my research is to investigate cyanide as a metabolic toxin and develop novel therapies for cyanide toxicity, a hazardous environmental pollutant and potential biological warfare agent.

- Research Assistant (Jan 2019 – Aug 2019) with Dr. Lolitika Mandal at Department of Biological Sciences at Indian Institute of Science Education and Research (IISER) Mohali, India. Research goals: *Mitochondrial fatty acid oxidation regulates state and fate of hematopoietic progenitors in Drosophila.*

- **PhD** in Department of Biological Sciences at Indian Institute of Science Education and Research (IISER) Mohali, India, under the supervision of Dr. Sudip Mandal (August 2010 – Jan 2019). Thesis title: *Understanding mechanistic basis of regulation of cell growth by mitochondrial activity in Drosophila melanogaster.*

- **Master of Science** (M.Sc.) in ***Biotechnology*** from Swami Ramanand Tirth Marathwada University, Nanded (2007-2009), (CGPA-8.41). Thesis title: *Study of vacuolar serine proteases from Plasmodium falciparum and their interaction with Chalcones* under supervision of Prof. C N Khobragade.

- **Bachelor of Science** (B.Sc.) in ***Microbiology*** from Swami Ramanand Tirth Marathwada University, Nanded (2004-2007)

Publications

- **Toshniwal A**, Gupta S, Mandal L, and Mandal S. ROS inhibit cell growth by regulating 4EBP and S6K, independent of TOR during development. **Developmental Cell 49, 473-489.e9, May 06, 2019** https://doi.org/10.1016/j.devcel.2019.04.008

- Tiwari S, **Toshniwal A**, Mandal S, and Mandal L. Fatty acid β-oxidation regulates hemocyte progenitor homeostasis in *Drosophila* larval lymph gland ***(Manuscript under Review)***.

Laboratory skills and Technical expertise

- **Molecular Biology Techniques** DNA isolation, RNA isolation, plasmid isolation, Gel electrophoresis, Cloning, RT-PCR, Real Time PCR, SDS-PAGE, Western Blotting.
- **Microbiological techniques** Microbial culture and general microbiological techniques, Transformation, cloning.
- **Microscopy** Confocal Microscopy (Zeiss LSM780, Leica SP8), Fluorescence (Zeiss Scope.A1, Zeiss Discovery.V8 and Zeiss LUMAR V12). Familiar with Image J.
- **Bioinformatics** Clustal W, Primer Designing, Basic sequence analysis.
- **Cytological Techniques** Immunohistochemistry, ROS assays, cell proliferation and cell death analysis assays, cell cycle analysis
- **Biochemical Techniques** Mitochondrial isolation, ATP assay, Protein estimation, Complex I activity of ETC, Citrate synthase assay and Acetyl CoA assay.

- **Cell culture**
- Histone isolation and western analysis.
- *Drosophila* genetics, dissection and Immunofluorescence analysis of larval tissues.
- Making single cell suspension from tissues and Cell cycle analysis by FACS.
- Statistical analysis with SigmaPlot and Graph Pad Prism

Well versed with imaging and image processing software » Axiovision, ZEN, Adobe Photoshop, ImageJ.

Awards and Fellowship

- **EMBO Travel grant** for attending EMBO- Size and Shape 2018, Bangalore, India.
- **International Travel grant** for attending the 18th International Meeting of Developmental Biology, Singapore 2017 by **Company of Biologist.**
- **ICMR- Senior Research Fellowship** (SRF) from **Indian Council of Medical & Research**, **Govt. of India**, Delhi, India 2012.
- Awarded **Junior Research Fellowship (JRF)** from University Grants Commision (**UGC**), India, June, 2011.
- **ICMR- Senior Research Fellowship** (SRF) from **Indian Council of Medical & Research**, **Govt. of India**, Delhi, India 2010.

Workshops and Symposium participated

- Oral presentation at **EMBO-Shape and Size** 2018, NCBS, Bangalore India. (2018)
- Oral presentation at **18th International Meeting of Developmental Biology, Singapore** 2017.
- Poster presentation at XXXVIII All India Cell Biology Conference and International Symposium on **'Cellular Response to Drugs'** held at CDRI, Lucknow, India. (2014)
- Poster presentation at **Mitochondria, Metabolism and Energetics 38th Mahabaleshwar Seminar** held at Mahabaleshwar India. (2014)
- Poster presentation at **Cells to Cellular Programming** Symposium organized by Wellcome trust-DBT India Alliance and IISER Mohali, India. (**2016**)

- Poster presentation at **India-EMBO Partnership Symposia** held at IISER Mohali, India. (**2016**)
- **Course on Advanced Microscopy and Imaging Techniques** Jointly organized by DSS Imagetech, Olympus and Photometrics, 28-30 May, **2013**.
- Poster presentation at **Indian Society of Developmental Biology Conference 2012.** conducted by IIT-Kanpur at Jaipur.
- Poster presentation at **The XXXV All India Cell Biology conference and symposium on Membrane dynamics and disease** held at NISER-Bhubaneshwar, India. (**2011**).

Interpersonal skills

I have been directly involved in research training of three master's students & several summer interns in my Ph.D. I have also served as teaching assistant for both undergraduate & postgraduate practical classes at IISER Mohali.
I have strong interpersonal and written and oral communication skills.
Good analytical and problem solving abilities. Good team leading skills.

References

Re: [EXTERNAL] Possible to amend permit application?

Elise Lauterbur <lauterbur@gmail.com>

Sun 2/2/2020 2:09 PM

To: Management Authority, FWHQ <ManagementAuthority@fws.gov>

2 attachments (88 KB)

CITES_import_application_2020-1-31.docx; CV Rajaonarivelo Tsiky 2019.pdf;

Dear FWS,

Thank you for your help in adding information to our application file. Because we have had to add an additional veterinarian to the sampling for scheduling reasons, as well as separate some samples from individual animals into more vials than expected, I have attached a new sample table that reflects those separated sample vials and the additional veterinarian's resume here.

Thanks again,
Elise

On Mon, Jan 13, 2020 at 5:51 AM Management Authority, FWHQ <managementauthority@fws.gov> wrote:

> Dear Elise,
>
> Thank you for the additional information. The additional information has been added to your application file.
>
> We appreciate your time and consideration.
>
> **********
>
> U.S. Fish and Wildlife Service
> International Affairs Program
> Division of Management Authority
> **Reply to: ManagementAuthority@fws.gov,** http://www.fws.gov/international/permits/
> ***Sign up for our e-newsletter to learn how we're working around the globe to protect species and their habitats!***
> **Going abroad? Be Informed. Buy Informed.**
> **********
> *Mailing address:*
> *ATTN DIVISION OF MANAGEMENT AUTHORITY - BRANCH OF PERMITS*
> *U.S. FISH & WILDLIFE SERVICE HEADQUARTERS*
> *5275 LEESBURG PIKE, MS: IA*
> *FALLS CHURCH, VA 22041-3803*
> Stamp out extinction with the Save Vanishing Species Stamp
>
> On Wed, Jan 8, 2020 at 1:12 PM Elise Lauterbur <lauterbur@gmail.com> wrote:
>
> > My apologies, also attached here is the CV of the additional collaborator.
> >
> > Thanks,
> > Elise

On Wed, Jan 8, 2020 at 11:09 AM Elise Lauterbur <lauterbur@gmail.com> wrote:

Dear FWS,

Thank you very much. I have attached additional information to this email in two formats - The first file, "CITES_import_application_2019_12_27.doc" is an updated version of the application as it was originally submitted, with the additional information included alongside the original information. To facilitate seeing the additional information, I have also attached "CITES_import_application_2019_12_27_additional.doc" which includes only the additional information, in the appropriate sections.

Thank you again for allowing us to add this additional information while the application is being reviewed.
Best,
Elise

On Thu, Dec 19, 2019 at 7:38 AM Management Authority, FWHQ <managementauthority@fws.gov> wrote:

Dear Eilse,

Thank you for your inquiry. You may submit additional information to this email to be added to your application file since your application is still being reviewed.

We appreciate your time and consideration.

****

U.S. Fish and Wildlife Service
International Affairs Program
Division of Management Authority
Branch of Permits
1-800-358-2104
Reply to: ManagementAuthority@fws.gov, http://www.fws.gov/international/permits/
***Sign up* for our e-newsletter to learn how we're working around the globe to protect species and their habitats!**
Going abroad? Be Informed. Buy Informed.
****
Mailing address:
ATTN DIVISION OF MANAGEMENT AUTHORITY - BRANCH OF PERMITS
U.S. FISH & WILDLIFE SERVICE HEADQUARTERS
5275 LEESBURG PIKE, MS: IA
FALLS CHURCH, VA 22041-3803
Stamp out extinction with the Save Vanishing Species Stamp

On Wed, Dec 11, 2019 at 11:36 AM Elise Lauterbur <lauterbur@gmail.com> wrote:

Good morning,

Your office is currently processing our permit application **US56547D/9, and I have just been notified that we would like to import some additional samples from the same individual animals. (Eg. Currently the permit requests blood samples for three species, we would also like to import skin biopsy samples from the same individuals from which

we will obtain the blood samples.)

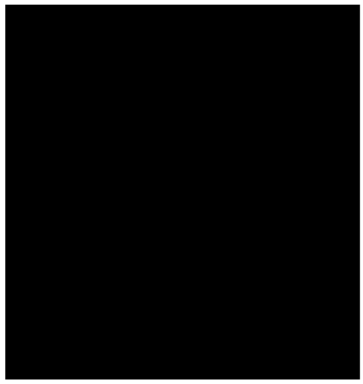
Could you please tell me if it is possible to amend this permit application, or should we prepare a completely new application for these additional samples?

Thank you,
Elise

Curriculum Vitae

RAJAONARIVELO Tsiky Hariniaina

[redacted]



EDUCATION

Depuis 2013	Docteur en Médecine Vétérinaire, ONDVM n°436 du 13-02-15
2004 - 2013	Université d'Ambohitsaina Antananarivo, Madagascar Faculté de Médecine, option Médecine Vétérinaire
2004	E.S.C.A (Ecole Sacrée Cœur Antanimena) ANTANANARIVO 101 Baccalauréat série D Scientifique

EXPERIENCES PROFESSIONNELS

Octobre 2019 à ce jour	Vétérinaire à temps partiel à la clinique Universitaire Vétérinaire Ambatobe, Antananarivo, Madagascar
Mars - Juin 2019	Stage interne vétérinaire au Duke Lemur Center, Caroline du nord, Etats Unis
Juin 2016 – Aout 2019	Vétérinaire à la clinique Vetclinic Animal SOS Ampandrianomby, Antananarivo, Madagascar
Février- Avril 2016	Stage vétérinaire au Tuxedo Animal Hospital, Birchwood Animal Hospital, Emergency Animal Hospital, Assiniboine Zoo and Park à Winnipeg, Canada
Janvier - Février 2016	Stage vétérinaire à la clinique Vetclinic, Analamahitsy, Antananarivo, Madagascar
Mars 2015 à ce jour	Vétérinaire à Madagascar Dog initiative Ranomafana Fianarantsoa, et Andasibe Moramanga, Madagascar
Septembre 2014-Janvier 2016	Assistant vétérinaire intermittent à la Clinique Vétomobile Sabotsy Namehana, Antananarivo, Madagascar
Septembre 2012-Septembre 2017	Assistant vétérinaire au Program of biomedical assessment of lemurs à Ambatovy forest dans Ambatovy Group and Duke Lemur Center Moramanga, Toamasina, Madagascar
28 Novembre – 2 Décembre 2011	Stage de Médecine au Laboratoire de Biologie Médicale (LBM) Antananarivo, Madagascar

21 – 25 Novembre 2011	Stage vétérinaire à la Clinique vétérinaire Lysanel, Andoharanofotsy, Antananarivo, Madagascar
24 - 28 Octobre 2011	Stage vétérinaire à la Clinique vétérinaire MIZAMI, Antananarivo, Madagascar
10 - 16 Octobre 2011	Stage vétérinaire élevage de vache laitière à Miarinarivo, Itasy, Madagascar
3 - 7 Octobre 2011	Stage vétérinaire élevage avicole Antananarivo, Madagascar
26 - 30 Septembre 2011	Stage vétérinaire à la Direction des Services Vétérinaires (DSV), Antananarivo, Madagascar
19 - 23 Septembre 2011	Stage vétérinaire d'hygiène et d'inspection des Denrées Alimentaires d'Origine Animal, Ampasika, Antananarivo, Madagascar
3 - 10 Août 2011	Stage vétérinaire au Laboratoire National de Diagnostic Vétérinaire (LNDV), Itaosy, Antananarivo, Madagascar
30 Décembre 2010	Volontaire et assistant vétérinaire à Carnivores of Madagascar
10 Janvier 2011	project EARTHWATCH INSTITUTE, Ankarafantsika, Madagascar
Mars - Décembre 2010	Stage de thèse en Médecine vétérinaire. Thèse intitulée: **Suivi nutritionnel de trois espèces de Lémuriens dans deux parcs zoologiques de Madagascar** au Parc Zoologique d'Ivoloina (PZI) Toamasina, Madagascar and au Parc Botanique et Zoologique de Tsimbazaza (PBZT), Antananarivo, Madagascar Directeur de thèse et encadreur : Pr RATSIMBAZAFY Henri Jonah et Pr RASAMBAINARIVO Jhon Henri
28 Août – 4 Septembre 2009	Stage vétérinaire à la Direction des Services Vétérinaires Region Menabe, region Bongolava, region Analanjirofo, region Alaotra Mangoro Research project on emerging disease in Indian Ocean en collaboration avec : Direction des Services Vétérinaires(DSV), Centre de Coopération International en Recherche Agronomique pour le Développement (CIRAD), Département de Recherches Zootechniques et Vétérinaires (FOFIFA-DRZV), Laboratoire National de Diagnostic Vétérinaire (LNDV), Département d'Enseignement des Sciences et de Médecine Vétérinaire (DESMV), program Animal Risk-OI
21 - 25 Août 2009	Stage vétérinaire à la clinique Lysanel Andoharanofotsy Antananarivo, Madagascar
12 - 13 Août 2009	Stage vétérinaire à la Ferme équestre Anosibe Antananarivo, Madagascar
18 - 21 Mai 2009	Stage vétérinaire au Parc Zoologique d'Ivoloina Toamasina, Atelier sur la Médecine des animaux de Parcs Zoologiques
24 Octobre - 16 Novembre 2008	Stage vétérinaire au Parc Zoologique d'Ivoloina Toamasina, Médecine de Zoo et Gestion en captivité de la Faune Sauvage de Madagascar
13 - 28 Mars 2008	Stage vétérinaire à la Ferme-Ecole Tombotsoa, Antsirabe, Madagascar
1 - 10 Novembre 2007	Stage de biologie à la forêt de Sahafina, Sous Préfecture de Brickaville, Madagascar

INTERET

Membre du : Groupe d'Etude et de Recherche sur les Primates (GERP)
Autres activités: [redacted]

"Je déclare sur l'honneur l'exactitude des renseignements me concernant ci-dessus »

L'intéressée,
Dr RAJAONARIVELO TSIKY HARINIAINA

Re: [EXTERNAL] Re: CITES Permit App 56547D

Elise Lauterbur <lauterbur@gmail.com>

Wed 4/8/2020 4:09 PM

To: Cate, Emily B <emily_cate@fws.gov>

5 attachments (2 MB)

Animals_involved_8_April_2020.xlsx; Sampling_updated_peranimal_8_April_2020.xlsx; CHANEY_approved_IACUC.pdf; Permit_Morgan_CHANEY_02012020.pdf; CITES permit app 56547D - April 2020.docx;

Hi Emily,

Great, thank you. I have attached a document answering your questions called "CITES permit app 56547D - April 2020.docx," two additional spreadsheets for questions 2 and 3, and two additional files (updated IACUC and a research permit that was granted after our initial submission). I've included an additional statement on how these activities will benefit the wild *Eulemur rufifrons* population, as well as why *E. rufifrons* samples will benefit *H. aureus* and *P. simus* populations - please let me know if you need more detail.

Thanks again!
Elise

On Tue, Apr 7, 2020 at 10:37 AM Cate, Emily B <emily_cate@fws.gov> wrote:

> **Hi Elise,**
>
> **Thank you for gathering up this information. I would prefer to have it as one row per animal sampled (multiple samples per animal okay). Please also include a count of the total number of animals and let me know if you have any questions.**
>
> **Regards,**
> **Emily**
>
> ---
>
> **From:** Elise Lauterbur <lauterbur@gmail.com>
> **Sent:** Monday, April 6, 2020 5:48 PM
> **To:** Cate, Emily B <emily_cate@fws.gov>
> **Subject:** [EXTERNAL] Re: CITES Permit App 56547D
>
> Dear Emily,
>
> Thank you for your email, and for working on processing our application. I'm working on the answers to your questions - for the second question (updated table of samples to reflect January and planned February/March sampling), would you like this as a table with one row per animal sampled (in which case parts c and f would include multiple samples, eg. c. 2 blood and 1 skin biopsy samples, f. 1 9ml vial with blood in buffer, 1 2ml vial with blood, 1 vial with skin biopsy), or as previously submitted with one row per specimen type?
>
> Thank you,

Elise

On Mon, Mar 30, 2020 at 7:33 AM Cate, Emily B <emily_cate@fws.gov> wrote:

Dear Ms. Lauterbur,

I have the application dated 09/26/2019, received 10/02/2019, regarding the proposed import of scientific samples collected from multiple species of lemurs in Madagascar. I apologize for the delay in processing your application.

Please provide the following information so that I may continue to process your application:

1. Provide the name and address of the exporter in the foreign country. (Name, Address, City, State/Province, Country, Postal Code)

2. Generally, did the proposed sampling during January 2020, as described in the application, occur? If so, please provide an updated answer for the following question:

For **each** animal/specimen involved in the proposed activity provide: a) Scientific Name, b) Common name, c) Number and type of sample/part, d) Wild or captive born, E) approximate date of collection (mm/yyyy), f) Description of packaging (e.g., vials, slides, envelopes, etc.), and g) Total number of all samples in the shipment.

3. If the proposed January 2020 sampling occurred, please provide an updated answer to the following if any differences occurred than what was originally submitted in the application:

For **each** animal/specimen involved in the proposed activity provide: a) scientific name and common name, b) specific location of where, when, and by whom (name and address) the specimen was removed from the wild, c) Purpose of removal and length or approximate length of time held in captivity. Discuss issues such as the method of collection, was the collection done as part of a larger study, were animals returned to the wild after sampling, and did any mortalities or injuries occur due to collection or holding, d) If and how any remuneration, either financial or in-kind, was provided for taking or capturing animals or for the collection of samples, e) Your efforts to use captive specimens (e.g., captive-born, captive-held), or parts thereof, in lieu of taking animals from the wild, f) Copies of your foreign or domestic collecting permit, license, contract, or agreement, g) Documentation showing that the specimen(s) was/were legally obtained by the applicant, and h) Copies of any applicable State, Tribal, Federal, or Foreign government permits or licenses that authorized the removal of this animal from the wild.

4. Please clarify the proposed necessary use of red-fronted brown lemurs (*Eulemur rufus*) in the study. The doctoral dissertation submitted with the application states that captive-held ring-tailed lemurs (*Lemur catta*) will be used and the corresponding IACUC submitted did not include the use of red-fronted brown lemurs. In addition, the answer to question 10(a) made reference to the brown lemur (*Eulemur fulvus*), while a reference to the red-fronted brown lemur was absent. Please clarify if there are samples proposed to be imported from the brown lemur as well. **Please note that for each species, we will need a statement on how the activities will enhance or benefit the wild population** (I see this answered for only the greater bamboo lemur (*Prolemur simus*) and the golden bamboo lemur (*Hapalemur aureus*)). If you have questions about this requirement, please let me know, as it may be easier to move forward by having separate applications for the species.

Please submit the requested information in one submission directly to me via email (attachments such as spreadsheets describing the multiple samples are encouraged). Please let me know if you have any questions or concerns.

In accordance with 50 CFR 13 11(e), if the requested information is not received by this office by **May 14, 2020**, your application will be abandoned and administratively closed. Once a file is closed you will need to submit a new application and all required fees for the Service to consider your proposed activity Please refer to permit number 56547D in your correspondence.

Respectfully,
Emily

Emily Cate | Permits Biologist
U.S. Fish and Wildlife Service | International Affairs
Division of Management Authority | Branch of Permits
5725 Leesburg Pike, MS:IA
Falls Church, VA 22041-3803

a. scientific and common name	b. specific location of where, when, and by whom removed from the wild	c. purpose of removal and length of time in captivity	method of collection	collection part of larger study	returned to wild after sampling	mortalities/injuries
Prolemur simus , Greater bamboo lemur	Where: Karianga, Ivato, District Vondrozo, Madagascar When: 1 August 2019; 2020, exact date TBD once COVID-19 danger has passed Whom: Dr. Patricia C. Wright	2019: Collaring to prepare for translocation, 5 hours captive 2020: Translocation, has not yet occurred, expected time captive 48 hours	Telazol injection	Yes, translocation of a family group from unprotected to protected habitat	yes	none
Prolemur simus , Greater bamboo lemur	Where: Karianga, Ivato, District Vondrozo, Madagascar When: 1 August 2019; 2020, exact date TBD once COVID-19 danger has passed Whom: Dr. Patricia C. Wright	2019: Collaring to prepare for translocation, 5 hours captive 2020: Translocation, has not yet occurred, expected time captive 48 hours	Telazol injection	Yes, translocation of a family group from unprotected to protected habitat	yes	none
Prolemur simus , Greater bamboo lemur	Where: Karianga, Ivato, District Vondrozo, Madagascar When: 2 August 2019; 2020, exact date TBD once COVID-19 danger has passed Whom: Dr. Patricia C. Wright	2019: Collaring to prepare for translocation, 5 hours captive 2020: Translocation, has not yet occurred, expected time captive 48 hours	Telazol injection	Yes, translocation of a family group from unprotected to protected habitat	yes	none
Prolemur simus , Greater bamboo lemur	Where: Karianga, Ivato, District Vondrozo, Madagascar When: 2 August 2019; 2020, exact date TBD once COVID-19 danger has passed Whom: Dr. Patricia C. Wright	2019: Collaring to prepare for translocation, 5 hours captive 2020: Translocation, has not yet occurred, expected time captive 48 hours	Telazol injection	Yes, translocation of a family group from unprotected to protected habitat	yes	none
Prolemur simus , Greater bamboo lemur	Where: Karianga, Ivato, District Vondrozo, Madagascar When: 2 August 2019; 2020, exact date TBD once COVID-19 danger has passed Whom: Dr. Patricia C. Wright	2019: Collaring to prepare for translocation, 4 hours captive 2020: Translocation, has not yet occurred, expected time captive 48 hours	Telazol injection	Yes, translocation of a family group from unprotected to protected habitat	yes	none
Prolemur simus , Greater bamboo lemur	Where: Karianga, Ivato, District Vondrozo, Madagascar When: 2 August 2019; 2020, exact date TBD once COVID-19 danger has passed Whom: Dr. Patricia C. Wright	2019: Collaring to prepare for translocation, 7 hours captive 2020: Translocation, has not yet occurred, expected time captive 48 hours	Telazol injection	Yes, translocation of a family group from unprotected to protected habitat	yes	none
Prolemur simus , Greater bamboo lemur	Where: Karianga, Ivato, District Vondrozo, Madagascar When: 2 August 2019; 2020, exact date TBD once COVID-19 danger has passed Whom: Dr. Patricia C. Wright	2019: Collaring to prepare for translocation, 7 hours captive 2020: Translocation, has not yet occurred, expected time captive 48 hours	Telazol injection	Yes, translocation of a family group from unprotected to protected habitat	yes	none
Prolemur simus , Greater bamboo lemur	Where: Karianga, Ivato, District Vondrozo, Madagascar When: 2 August 2019; 2020, exact date TBD once COVID-19 danger has passed Whom: Dr. Patricia C. Wright	2019: Collaring to prepare for translocation, 7 hours captive 2020: Translocation, has not yet occurred, expected time captive 48 hours	Telazol injection	Yes, translocation of a family group from unprotected to protected habitat	yes	none
Prolemur simus , Greater bamboo lemur	Where: Karianga, Ivato, District Vondrozo, Madagascar When: 3 August 2019; 2020, exact date TBD once COVID-19 danger has passed Whom: Dr. Patricia C. Wright	2019: Collaring to prepare for translocation, 7 hours captive 2020: Translocation, has not yet occurred, expected time captive 48 hours	Telazol injection	Yes, translocation of a family group from unprotected to protected habitat	yes	none
Prolemur simus , Greater bamboo lemur	Where: Karianga, Ivato, District Vondrozo, Madagascar When: 3 August 2019; 2020, exact date TBD once COVID-19 danger has passed Whom: Dr. Patricia C. Wright	2019: Collaring to prepare for translocation, 7 hours captive 2020: Translocation, has not yet occurred, expected time captive 48 hours	Telazol injection	Yes, translocation of a family group from unprotected to protected habitat	yes	none
Prolemur simus , Greater bamboo lemur	Where: Karianga, Ivato, District Vondrozo, Madagascar When: 3 August 2019; 2020, exact date TBD once COVID-19 danger has passed Whom: Dr. Patricia C. Wright	2019: Collaring to prepare for translocation, 7 hours captive 2020: Translocation, has not yet occurred, expected time captive 48 hours	Telazol injection	Yes, translocation of a family group from unprotected to protected habitat	yes	none
Prolemur simus , Greater bamboo lemur	Where: Karianga, Ivato, District Vondrozo, Madagascar When: 3 August 2019; 2020, exact date TBD once COVID-19 danger has passed Whom: Dr. Patricia C. Wright	2019: Collaring to prepare for translocation, 7 hours captive 2020: Translocation, has not yet occurred, expected time captive 48 hours	Telazol injection	Yes, translocation of a family group from unprotected to protected habitat	yes	none
Hapalemur aureus, Golden bamboo lemur	Where: Ranomafana National Park, Districts Haute Matsiatra and Vatovavy-Fitovinany, Madagascar When: 18 Jan 2020 Whom: Morgan Chaney	Obtaining samples, 4 hours captive	Telazol injection	Yes, study of dietary cyanide adaptation and conservation genetics	yes	none
Hapalemur aureus, Golden bamboo lemur	Where: Ranomafana National Park, Districts Haute Matsiatra and Vatovavy-Fitovinany, Madagascar When: 18 Jan 2020 Whom: Morgan Chaney	Obtaining samples, 3 hours captive	Telazol injection	Yes, study of dietary cyanide adaptation and conservation genetics	yes	none

Hapalemur aureus, Golden bamboo lemur	Where: Ranomafana National Park, Districts Haute Matsiatra and Vatovavy-Fitovinany, Madagascar When: 18 Jan 2020 Whom: Morgan Chaney	Obtaining samples, 4 hours captive	Telazol injection	Yes, study of dietary cyanide adaptation and conservation genetics	yes	none
Hapalemur aureus, Golden bamboo lemur	Where: Ranomafana National Park, Districts Haute Matsiatra and Vatovavy-Fitovinany, Madagascar When: 19 Jan 2020 Whom: Morgan Chaney	Obtaining samples, 5 hours captive	Telazol injection	Yes, study of dietary cyanide adaptation and conservation genetics	yes	none
Hapalemur aureus, Golden bamboo lemur	Where: Ranomafana National Park, Districts Haute Matsiatra and Vatovavy-Fitovinany, Madagascar When: 19 Jan 2020 Whom: Morgan Chaney	Obtaining samples, 5 hours captive	Telazol injection	Yes, study of dietary cyanide adaptation and conservation genetics	yes	none
Hapalemur aureus, Golden bamboo lemur	Where: Ranomafana National Park, Districts Haute Matsiatra and Vatovavy-Fitovinany, Madagascar When: 19 Jan 2020 Whom: Morgan Chaney	Obtaining samples, 5 hours captive	Telazol injection	Yes, study of dietary cyanide adaptation and conservation genetics	yes	none
Hapalemur aureus, Golden bamboo lemur	Where: Ranomafana National Park, Districts Haute Matsiatra and Vatovavy-Fitovinany, Madagascar When: 19 Jan 2020 Whom: Morgan Chaney	Obtaining samples, 5 hours captive	Telazol injection	Yes, study of dietary cyanide adaptation and conservation genetics	yes	none
Hapalemur aureus, Golden bamboo lemur	Where: Ranomafana National Park, Districts Haute Matsiatra and Vatovavy-Fitovinany, Madagascar When: 19 Jan 2020 Whom: Morgan Chaney	Individual found dead in National Park in 2016	Necropsy	No	N/A	Found dead
Eulemur rufifrons , Red-fronted brown lemur	Where: Ranomafana National Park, Districts Haute Matsiatra and Vatovavy-Fitovinany, Madagascar When: 20 Jan 2020 Whom: Morgan Chaney	Obtaining samples, 4 hours captive	Telazol injection	Yes, study of dietary cyanide adaptation and conservation genetics	yes	none
Eulemur rufifrons , Red-fronted brown lemur	Where: Ranomafana National Park, Districts Haute Matsiatra and Vatovavy-Fitovinany, Madagascar When: 20 Jan 2020 Whom: Morgan Chaney	Obtaining samples, 3 hours captive	Telazol injection	Yes, study of dietary cyanide adaptation and conservation genetics	yes	none
Eulemur rufifrons , Red-fronted brown lemur	Where: Ranomafana National Park, Districts Haute Matsiatra and Vatovavy-Fitovinany, Madagascar When: 20 Jan 2020 Whom: Morgan Chaney	Obtaining samples, 3 hours captive	Telazol injection	Yes, study of dietary cyanide adaptation and conservation genetics	yes	none
Hapalemur aureus, Golden bamboo lemur	Where: Ranomafana National Park, Districts Haute Matsiatra and Vatovavy-Fitovinany, Madagascar When: 2020, exact date TBD once COVID-19 danger has passed Whom: Dr. Patricia C. Wright	Obtaining samples, has not yet occurred, expected time captive < 5 hours	Telazol injection	Yes, study of dietary cyanide adaptation and conservation genetics	yes	capture has not yet taken place
Hapalemur aureus, Golden bamboo lemur	Where: Ranomafana National Park, Districts Haute Matsiatra and Vatovavy-Fitovinany, Madagascar When: 2020, exact date TBD once COVID-19 danger has passed Whom: Dr. Patricia C. Wright	Obtaining samples, has not yet occurred, expected time captive < 5 hours	Telazol injection	Yes, study of dietary cyanide adaptation and conservation genetics	yes	capture has not yet taken place
Hapalemur aureus, Golden bamboo lemur	Where: Ranomafana National Park, Districts Haute Matsiatra and Vatovavy-Fitovinany, Madagascar When: 2020, exact date TBD once COVID-19 danger has passed Whom: Dr. Patricia C. Wright	Obtaining samples, has not yet occurred, expected time captive < 5 hours	Telazol injection	Yes, study of dietary cyanide adaptation and conservation genetics	yes	capture has not yet taken place
Hapalemur aureus, Golden bamboo lemur	Where: Ranomafana National Park, Districts Haute Matsiatra and Vatovavy-Fitovinany, Madagascar When: 2020, exact date TBD once COVID-19 danger has passed Whom: Dr. Patricia C. Wright	Obtaining samples, has not yet occurred, expected time captive < 5 hours	Telazol injection	Yes, study of dietary cyanide adaptation and conservation genetics	yes	capture has not yet taken place
Hapalemur aureus, Golden bamboo lemur	Where: Ranomafana National Park, Districts Haute Matsiatra and Vatovavy-Fitovinany, Madagascar When: 2020, exact date TBD once COVID-19 danger has passed Whom: Dr. Patricia C. Wright	Obtaining samples, has not yet occurred, expected time captive < 5 hours	Telazol injection	Yes, study of dietary cyanide adaptation and conservation genetics	yes	capture has not yet taken place
Hapalemur aureus, Golden bamboo lemur	Where: Ranomafana National Park, Districts Haute Matsiatra and Vatovavy-Fitovinany, Madagascar When: 2020, exact date TBD once COVID-19 danger has passed Whom: Dr. Patricia C. Wright	Obtaining samples, has not yet occurred, expected time captive < 5 hours	Telazol injection	Yes, study of dietary cyanide adaptation and conservation genetics	yes	capture has not yet taken place
Eulemur rufifrons , Red-fronted brown lemur	Where: Ranomafana National Park, Districts Haute Matsiatra and Vatovavy-Fitovinany, Madagascar When: 2020, exact date TBD once COVID-19 danger has passed Whom: Dr. Patricia C. Wright	Obtaining samples, has not yet occurred, expected time captive < 5 hours	Telazol injection	Yes, study of dietary cyanide adaptation and conservation genetics	yes	capture has not yet taken place

Eulemur rufifrons , Red-fronted brown lemur	Where: Ranomafana National Park, Districts Haute Matsiatra and Vatovavy-Fitovinany, Madagascar When: 2020, exact date TBD once COVID-19 danger has passed Whom: Dr. Patricia C. Wright	Obtaining samples, has not yet occurred, expected time captive < 5 hours	Telazol injection	Yes, study of dietary cyanide adaptation and conservation genetics	yes	capture has not yet taken place
Eulemur rufifrons , Red-fronted brown lemur	Where: Ranomafana National Park, Districts Haute Matsiatra and Vatovavy-Fitovinany, Madagascar When: 2020, exact date TBD once COVID-19 danger has passed Whom: Dr. Patricia C. Wright	Obtaining samples, has not yet occurred, expected time captive < 5 hours	Telazol injection	Yes, study of dietary cyanide adaptation and conservation genetics	yes	capture has not yet taken place
Eulemur rufifrons , Red-fronted brown lemur	Where: Ranomafana National Park, Districts Haute Matsiatra and Vatovavy-Fitovinany, Madagascar When: 2020, exact date TBD once COVID-19 danger has passed Whom: Dr. Patricia C. Wright	Obtaining samples, has not yet occurred, expected time captive < 5 hours	Telazol injection	Yes, study of dietary cyanide adaptation and conservation genetics	yes	capture has not yet taken place
Eulemur rufifrons , Red-fronted brown lemur	Where: Ranomafana National Park, Districts Haute Matsiatra and Vatovavy-Fitovinany, Madagascar When: 2020, exact date TBD once COVID-19 danger has passed Whom: Dr. Patricia C. Wright	Obtaining samples, has not yet occurred, expected time captive < 5 hours	Telazol injection	Yes, study of dietary cyanide adaptation and conservation genetics	yes	capture has not yet taken place

a. Scientific name	b. Common name	c. Number and type of sample/part	d. Wild or captive-born	e. Approximate date of collection	f. Description of packaging	Birth date	Sex	Permanent markings
Prolemur simus	Greater Bamboo Lemur	4 blood; 1 buffy coat; 1 hair; 1 plasma; 2 protein	Wild	1 Aug 2019	Blood (2mL tubes); buffy coat (2 mL tube); hair (plucked, 2mL tube); plasma (2mL tube); protein saver card	Estimated 2018	Male	Microchip #0A01750051
Prolemur simus	Greater Bamboo Lemur	4 blood; 1 buffy coat; 1 hair; 1 plasma; 3 protein	Wild	1 Aug 2019	Blood (2mL tubes); buffy coat (2 mL tube); hair (plucked, 2mL tube); plasma (2mL tube); protein saver card	Unknown (adult)	Female	Microchip #0A02011231
Prolemur simus	Greater Bamboo Lemur	3 blood; 1 buffy coat; 1 hair; 1 plasma; 2 protein	Wild	2 Aug 2019	Blood (2mL tubes); buffy coat (2 mL tube); hair (plucked, 2mL tube); plasma (2mL tube); protein saver card	Unknown (subadult)	Male	Microchip #0A01750201
Prolemur simus	Greater Bamboo Lemur	3 blood; 1 buffy coat; 1 hair; 1 plasma; 2 protein	Wild	2 Aug 2019	Blood (2mL tubes); buffy coat (2 mL tube); hair (plucked, 2mL tube); plasma (2mL tube); protein saver card	Unknown (adult)	Female	Microchip #0A01752151
Prolemur simus	Greater Bamboo Lemur	3 blood; 1 buffy coat; 1 hair; 1 plasma; 2 protein	Wild	2 Aug 2019	Blood (2mL tubes); buffy coat (2 mL tube); hair (plucked, 2mL tube); plasma (2mL tube); protein saver card	Unknown (subadult)	Female	Microchip #0A01750829
Prolemur simus	Greater Bamboo Lemur	3 blood; 1 buffy coat; 1 hair; 1 plasma; 2 protein	Wild	2 Aug 2019	Blood (2mL tubes); buffy coat (2 mL tube); hair (plucked, 2mL tube); plasma (2mL tube); protein saver card	Unknown (subadult)	Female	Microchip #0A01752351
Prolemur simus	Greater Bamboo Lemur	3 blood; 1 buffy coat; 1 hair; 1 plasma; 2 protein	Wild	2 Aug 2019	Blood (2mL tubes); buffy coat (2 mL tube); hair (plucked, 2mL tube); plasma (2mL tube); protein saver card	Unknown (subadult)	Male	Microchip #0A01751807
Prolemur simus	Greater Bamboo Lemur	3 blood; 1 buffy coat; 1 hair; 1 plasma; 2 protein	Wild	2 Aug 2019	Blood (2mL tubes); buffy coat (2 mL tube); hair (plucked, 2mL tube); plasma (2mL tube); protein saver card	Unknown (adult)	Male	Microchip #0A01750603
Prolemur simus	Greater Bamboo Lemur	5 blood; 1 buffy coat; 1 hair; 1 plasma; 3 protein	Wild	3 Aug 2019	Blood (2mL tubes); buffy coat (2 mL tube); hair (plucked, 2mL tube); plasma (2mL tube); protein saver card	Unknown (adult)	Female	Microchip #0A01751302
Prolemur simus	Greater Bamboo Lemur	4 blood; 1 buffy coat; 1 hair; 1 plasma; 2 protein	Wild	3 Aug 2019	Blood (2mL tubes); buffy coat (2 mL tube); hair (plucked, 2mL tube); plasma (2mL tube); protein saver card	Unknown (adult)	Female	Microchip #0A01750920
Prolemur simus	Greater Bamboo Lemur	4 blood; 1 buffy coat; 1 hair; 1 plasma; 2 protein	Wild	3 Aug 2019	Blood (2mL tubes); buffy coat (2 mL tube); hair (plucked, 2mL tube); plasma (2mL tube); protein saver card	Unknown (adult)	Male	Microchip #0A01751109

Prolemur simus	Greater Bamboo Lemur	4 blood; 2 buffy coat; 1 hair; 1 plasma; 2 protein	Wild	3 Aug 2019	Blood (2mL tubes); buffy coat (2 mL tube); hair (plucked, 2mL tube); plasma (2mL tube); protein saver card	Unknown (adult)	Male	Microchip #0A01752527
Hapalemur aureus	Golden Bamboo Lemur	1 blood; 2 blood/serum	Wild	18 Jan 2020	Blood in buffer (9mL tube: 3mL blood, 6mL buffer); blood/serum (2mL tube)	Unknown (adult)	Male	Microchip #0A02010619
Hapalemur aureus	Golden Bamboo Lemur	1 blood; 2 blood/serum	Wild	18 Jan 2020	Blood in buffer (9mL tube: 3mL blood, 6mL buffer); blood/serum (2mL tube)	Unknown (adult)	Female	Microchip #0A02010639
Hapalemur aureus	Golden Bamboo Lemur	2 blood/serum	Wild	18 Jan 2020	Blood/serum (2mL tube)	Unknown (adult)	Female	Microchip #0A02011739
Hapalemur aureus	Golden Bamboo Lemur	1 blood; 2 blood/serum	Wild	19 Jan 2020	Blood in buffer (9mL tube: 3mL blood, 6mL buffer); blood/serum (2mL tube)	Unknown (adult)	Male	Microchip #0A02010713
Hapalemur aureus	Golden Bamboo Lemur	1 blood; 2 blood/serum	Wild	19 Jan 2020	Blood in buffer (9mL tube: 3mL blood, 6mL buffer); blood/serum (2mL tube)	Unknown (adult)	Female	Microchip #0A02010855
Hapalemur aureus	Golden Bamboo Lemur	1 blood; 2 blood/serum	Wild	19 Jan 2020	Blood in buffer (9mL tube: 3mL blood, 6mL buffer); blood/serum (2mL tube)	Unknown (adult)	Male	Microchip #0A02007860
Hapalemur aureus	Golden Bamboo Lemur	1 blood; 2 blood/serum	Wild	19 Jan 2020	Blood in buffer (9mL tube: 3mL blood, 6mL buffer); blood/serum (2mL tube)	Unknown (adult)	Female	Microchip #0A02035651
Eulemur rufifrons	Red-fronted brown lemur	1 blood; 2 blood/serum	Wild	20 Jan 2020	Blood in buffer (9mL tube: 3mL blood, 6mL buffer); blood/serum (2mL tube)	Unknown (adult)	Male	Microchip #0A02011754
Eulemur rufifrons	Red-fronted brown lemur	1 blood; 2 blood/serum	Wild	20 Jan 2020	Blood in buffer (9mL tube: 3mL blood, 6mL buffer); blood/serum (2mL tube)	Unknown (adult)	Male	Microchip #0A02007965
Eulemur rufifrons	Red-fronted brown lemur	1 blood; 2 blood/serum	Wild	20 Jan 2020	Blood in buffer (9mL tube: 3mL blood, 6mL buffer); blood/serum (2mL tube)	Unknown (adult)	Male	Microchip #0A02024118
Hapalemur aureus	Golden Bamboo Lemur	3 tissue samples	Wild	Found dead by local research technician, January 2016	Tissue (15mL tube)	Infant	Male	Preserved in freezer at Centre ValBio Madagascar
Eulemur rufifrons	Red-fronted brown lemur	1 blood; 2 blood/serum	Wild	Planned, post-COVID-19	Blood in buffer (9mL tube: 3mL blood, 6mL buffer); blood/serum (2mL tube)	Unknown (adult)	Unknown	Will be microchipped
Eulemur rufifrons	Red-fronted brown lemur	1 blood; 2 blood/serum	Wild	Planned, post-COVID-19	Blood in buffer (9mL tube: 3mL blood, 6mL buffer); blood/serum (2mL tube)	Unknown (adult)	Unknown	Will be microchipped
Eulemur rufifrons	Red-fronted brown lemur	1 blood; 2 blood/serum	Wild	Planned, post-COVID-19	Blood in buffer (9mL tube: 3mL blood, 6mL buffer); blood/serum (2mL tube)	Unknown (adult)	Unknown	Will be microchipped

Eulemur rufifrons	Red-fronted brown lemur	1 blood; 2 blood/serum	Wild	Planned, post-COVID-19	Blood in buffer (9mL tube: 3mL blood, 6mL buffer); blood/serum (2mL tube)	Unknown (adult)	Unknown	Will be microchipped
Eulemur rufifrons	Red-fronted brown lemur	1 blood; 2 blood/serum	Wild	Planned, post-COVID-19	Blood in buffer (9mL tube: 3mL blood, 6mL buffer); blood/serum (2mL tube)	Unknown (adult)	Unknown	Will be microchipped
Hapalemur aureus	Golden Bamboo Lemur	1 cell line from blood/plasma, 1 cell line from skin biopsy	Wild	Planned, post-COVID-19	2mL tube	Unknown (adult)	Unknown	Will be microchipped
Hapalemur aureus	Golden Bamboo Lemur	1 cell line from blood/plasma, 1 cell line from skin biopsy	Wild	Planned, post-COVID-19	2mL tube	Unknown (adult)	Unknown	Will be microchipped
Hapalemur aureus	Golden Bamboo Lemur	1 cell line from blood/plasma, 1 cell line from skin biopsy	Wild	Planned, post-COVID-19	2mL tube	Unknown (adult)	Unknown	Will be microchipped
Hapalemur aureus	Golden Bamboo Lemur	1 cell line from blood/plasma, 1 cell line from skin biopsy	Wild	Planned, post-COVID-19	2mL tube	Unknown (adult)	Unknown	Will be microchipped
Hapalemur aureus	Golden Bamboo Lemur	1 cell line from blood/plasma, 1 cell line from skin biopsy	Wild	Planned, post-COVID-19	2mL tube	Unknown (adult)	Unknown	Will be microchipped
Hapalemur aureus	Golden Bamboo Lemur	1 cell line from blood/plasma, 1 cell line from skin biopsy	Wild	Planned, post-COVID-19	2mL tube	Unknown (adult)	Unknown	Will be microchipped
Prolemur simus	Greater Bamboo Lemur	1 cell line from blood/plasma, 1 cell line from skin biopsy	Wild	Planned, post-COVID-19	2mL tube	Unknown (adult)	Male	Microchip #0A01751109 (to be translocated)
Prolemur simus	Greater Bamboo Lemur	1 cell line from blood/plasma, 1 cell line from skin biopsy	Wild	Planned, post-COVID-19	2mL tube	Unknown (adult)	Male	Microchip #0A01752527 (to be translocated)
Prolemur simus	Greater Bamboo Lemur	1 cell line from blood/plasma, 1 cell line from skin biopsy	Wild	Planned, post-COVID-19	2mL tube	Unknown (adult)	Male	Microchip #0A01750603 (to be translocated)

Prolemur simus	Greater Bamboo Lemur	1 cell line from blood/plasma, 1 cell line from skin biopsy	Wild	Planned, post-COVID-19	2mL tube	Unknown (adult)	Female	One of the 4 adult females to be translocated (sampled Aug 2019, see above)
Prolemur simus	Greater Bamboo Lemur	1 cell line from blood/plasma, 1 cell line from skin biopsy	Wild	Planned, post-COVID-19	2mL tube	Unknown (adult)	Female	One of the 4 adult females to be translocated (sampled Aug 2019, see above)
Prolemur simus	Greater Bamboo Lemur	1 cell line from blood/plasma, 1 cell line from skin biopsy	Wild	Planned, post-COVID-19	2mL tube	Unknown (adult)	Female	One of the 4 adult females to be translocated (sampled Aug 2019, see above)

1. The exporter in Madagascar is Dr. Benjamin Andriamihaja, MICET, Lot VU 283 D – Manakambahiny, 101 - ANTANANARIVO, Madagascar

2. Some of the proposed January 2020 sampling occurred. That which was amended for February and March 2020 in my January 31 email to FWS did not occur due to COVID-19, and is now planned to be carried out once the COVID-19 pandemic abates and sampling is again safe for both the researchers and the animals. (Safety for the animals will be determined by Madagascar National Parks, as well as the veterinarian involved.) Please see attached spreadsheet, "Sampling_updated_peranimal_8_April_2020.xlsx."

- Total number of animals sampled: 34
- Total number of *Prolemur simus* sampled: 12
  - The spreadsheet "Sampling_updated_peranimal_8_April_2020.xlsx" includes 18 *P. simus* rows. This is because, while only 12 animals have been/will be sampled, 6 of these will have additional samples taken when the family group is re-darted for their planned translocation. These are left as separate rows in the spreadsheet for clarity.
- Total number of *Hapalemur aureus* sampled: 14
- Total number of *Eulemur rufifrons* sampled: 8

3. Since some of the proposed sampling occurred, I am attaching a spreadsheet "Animals_involved_8_April_2020.xlsx" to clarify these sampling activities. The spreadsheet includes scientific and common names, specific locations of where, when, and by whom the animals were removed from (and returned to) the wild, the purpose and length of time held in captivity, details on larger studies, and mortalities/injuries (none). It also includes this information for the sampling described in the application that has not yet occurred because of COVID-19. Address details, purpose of removal, remuneration, and efforts to use captive specimens remain the same (copied below). Permit details remain the same except that the permit for sampling *Hapalemur aureus* by Morgan Chaney was granted, and is attached under the name "Permit_Morgan_CHANEY_02012020.pdf". (Please note that Madagascar uses the Day-Month-Year dating convention.)

b. Specific location of where, when, and by whom (name and address) the specimen was removed from the wild:

Species: *Prolemur simus*
Where: Karianga, Ivato, District Vondrozo, Madagascar
When: Aug. 1 – 3 2019. Second sampling planned for 2020 during translocation.
By whom: Dr. Patricia C. Wright, N-203 Social and Behavioral Sciences Building, Stony Brook University, Stony Brook NY 11794-4310, USA

Species: *Hapalemur aureus* and *Eulemur rufifrons*
Where: Ranomafana National Park, Districts Haute Matsiatra and Vatovavy-Fitovinany, Madagascar
When: January 18-20 2020, additional sampling planned for 2020 post-COVID-19.
By whom: Morgan Chaney, 750 Hilltop Drive, Room 226, Ken State University, Kent, Ohio 44242; Dr. Patricia C. Wright, N-203 Social and Behavioral Sciences Building, Stony Brook University, Stony Brook NY 11794-4310, USA

c. Purpose of removal and length of time held in captivity:

Prolemur simus:

Purpose: Animals were captured to fit them with radio collars and be examined by a veterinarian prior to a translocation from a disturbed region to a protected region, Ranomafana National Park. They will be re-captured in January of 2020 for the translocation. Human disturbance in their current range negatively affects the lemurs' food supply and has in the past included illegal poaching. In their new range they will be both protected from human disturbance and have the potential to contribute to the genetic diversity of the severely declining population in the protected region. The radio collars will provide the means to track the animals when they are released in the National Park. While they were anesthetized, blood samples and measurements were also taken for the analyses described below, including understanding the population genetics of the species. They will be re-darted once the COVID-19 pandemic has abated and translocated to Ranomafana National Park. At this time, additional samples (blood and skin biopsy for creating cell lines) will be taken from 6 adults.

Length of time: Each animal was held for approximately 5-8 hours between time of darting and time of release. They were released after full recovery from the effects of the anesthesia. When they are re-darted after the COVID-19 pandemic has abated, they will be held for 48 hours during translocation to Ranomafana National Park.

Hapalemur aureus and Eulemur rufifrons:
Purpose: Animals are being darted and captured for the purpose of obtaining blood samples for the research described below in question 10 and in detail in the attachment "DDRIG Proposal_Chaney.docx"

The skin biopsy will be taken for isolating fibroblasts. In short, the blood samples will be used to understand the genetics and biochemistry of cyanide adaptation at physiological levels, as well as conservation genetics. Both blood cells and skin cells will be used to examine whether cultured cells from these animals exhibit unusual tolerance to a high amount of cyanide. *H. aureus* eats 12-50 times the lethal dose for a similar weight animal, and *E. rufifrons* is a related species living in the same habitat that does not eat cyanide, which is important for providing a comparison.

Length of time: Each animal will be held for approximately 3-5 hours between time of darting and time of release. They were/will be released after full recovery from the effects of the anesthesia.

d. If and how any remuneration, either financial or in-kind, was provided for taking or capturing animals or for the collection of samples.

The supervising veterinarians, Dr. Hajanirina Rakotondrainibe and Tsiky Rajaonarivelo, was and will be paid a standard rate for their services in evaluating and monitoring the health of the lemurs. Darting technician Velotsara Jean Baptiste was and will be paid a standard rate for his services in darting the animals.

No remuneration of any kind was or will be provided for the samples themselves.

e. Your efforts to use captive specimens, or parts thereof, in lieu of taking animals from the wild:

The *Prolemur simus* samples are from animals that are part of a translocation program to transfer animals from a disturbed region to a protected region, to protect these individuals and the future genetic diversity of the species. Currently, the only population in a National Park consists of two related individuals, a father and daughter. Thus, to understand the genetic consequences of this translocation, the samples must come from the individuals translocated, and their capture for fitting radio collars and health examinations provided the opportunity to collect high quality DNA samples without additional disturbance. High quality samples, such as from blood, are imperative because additional conservation genetic studies will be carried out including analyses of nutritional and disease adaptations in this population.

The *Hapalemur aureus* samples are from wild animals eating their natural diet. There are no *H. aureus* currently living in captivity anywhere in the world. Thus, to understand anything about their population genetics and adaptation, including inbreeding, population connectivity, and nutritional adaptation, requires sampling wild individuals in their native range. The *Eulemur rufifrons* samples will be used to compare the nutritional adaptations of a non-bamboo eating species (*E. rufifrons*) with the bamboo specialized and cyanide adapted *H. aureus*. It is necessary that samples are taken from individuals that live in the same habitat and are exposed to the same dietary options as the *H. aureus* individuals sampled, thus we cannot use samples from captive *Eulemur.*

4. The references to *Eulemur fulvus* in sections 10a. and 10b. are typos using an outdated species designation, and should be *Eulemur rufifrons*, as in the rest of the application. (*Eulemur rufifrons* was previously considered a subspecies of *Eulemur fulvus,* as *Eulemur fulvus rufifrons*.)

In addition, the IACUC for Mr. Morgan Chaney previously submitted was incomplete. Please find attached "CHANEY_approved_IACUC.docx," which reflects the sampling of both *H. aureus* and *E. rufifrons.*

Regarding the use of *Lemur catta* as described in the doctoral dissertation proposal, while the use of captive *Lemur catta* was initially considered, the doctoral candidate and his committee determined that wild *Eulemur rufifrons* would be a much better comparison species. It is assumed that, across species, some variance in gene expression of the blood would be explainable by evolutionary relatedness. Like *Lemur catta, Eulemur rufifrons* is in the same taxonomic family as bamboo lemurs; therefore, we suspect that their use as a comparison will minimize the level of phylogenetically explainable variance in the statistical comparison of differentially expressed genes. *E. rufifrons* also lives in the same forests as *Hapalemur aureus,* whereas there are no free-ranging *L. catta* populations that are sympatric with *H. aureus.* In the context of this study, the shared habitat between *H. aureus* and *E. rufifrons* may mean that they are both exposed to similar types of parasites and seasonal stresses. These issues are important because they would certainly affect patterns of gene expression in the blood, and the comparison of these patterns is one of the primary goals of this study.

These samples will additionally provide two kinds of data that will directly benefit the wild *Eulemur rufifrons* population:

1. Data about the parasite load and diversity of these species. Because RNA will be extracted and sequenced from these samples, this will lead to some detection of bloodborne parasite-specific RNA in

addition to *E. rufifrons* RNA. These data will be used to estimate species of parasite (e.g., *Plasmodium* spp.) that are infecting these lemurs in the wild, potentially including infection load. Such data may be useful for future veterinary interventions and to aid in the restoration of reproductively depressed populations.

2. These samples can be used to build and refine *E. rufifrons* genomes for conservation genetics, including population demographic analyses to investigate the population history, heterozygosity, and potential inbreeding in *E. rufifrons.* Understanding these aspects of the population is important for continued conservation efforts. While species-level analyses of *E. rufifrons* have been conducted to understand their ancient population history, no population genetic analyses of *E. rufifrons* from this region have been conducted that could improve our understanding of how habitat fragmentation and other human influences have affected the population in the recent past. Similar studies have been conducted on the related *E. cinereiceps* (white-collared lemur), and have been usefully applied in the conservation of that species.

Lastly, the *E. rufifrons* samples will provide data that will benefit the captive and wild management of *H. aureus* and *P. simus* populations:

1. Wild management: *H. aureus* and *P. simus* are bamboo-dependent, and have naturally patchy distributions because they are restricted to forests with stands of woody bamboo, which itself has a naturally patchy distribution. An improved understanding of the dietary dependence of *H. aureus* and *P. simus* on woody bamboo, generated through comparison with the related species *E. rufifrons,* which shares forest habitat, could introduce new ways to successfully conserve them in protected areas. Current conservation efforts for these species include plans to propagate and re-establish stands of bamboo varieties that serve as food. Not only do they rely on specific species of bamboo (primarily *Cathariostachys madagascariensis*), they may rely on bamboo stands growing under conditions that result in specific nutrient profiles. *E. rufifrons* provides a non-bamboo dependent comparison for examining variation in gene expression attributable to bamboo diet vs. other factors.

2. Captive management: Bamboo lemurs, particularly *H. aureus,* have historically been challenging to successfully keep and breed in captivity, despite attempts to increase the population through captive breeding. This failure to thrive and breed in captivity could be a direct result of their dependence on specific bamboo species, and even those bamboo species grown under specific conditions. The gene expression adaptations of *H. aureus* and *P. simus* to the high concentrations of cyanide in their wild diet are likely to have secondary physiological effects, perhaps especially when cyanide is not present in their diet. A full understanding of these adaptations requires comparison of their gene expression patterns with those of a related species that is not cyanide adapted but shares habitat, *E. rufifrons*. Understanding their dietary requirements could improve captive care and breeding by providing captive individuals with more appropriate diets and nutrient profiles.

REQUEST TO USE ANIMALS – GENERAL INSTRUCTIONS

University of Akron
Kent State University
Northeast Ohio Medical University
Summa Health System
Youngstown State University

PROTOCOL COMPLETION

Each of the animal care programs at the institutions listed above uses the following “Request to Use Animals” (protocol) and the related "Annual Review" and “Modification” forms for all animal work involving live animals. Consult your Institutional Animal Care and Use Committee (IACUC) Coordinator to access the forms at your institution. Other forms may be required by each institution.

You must complete a new protocol form for each submission. Answer all questions that apply in a manner comprehensible to the layperson and define discipline specific terminology and abbreviations the first time they are used. Enter all responses in the answer boxes provided. For Yes/No questions and those that are not applicable (N/A), check the box or insert an “X” to the right of the appropriate response. Guidance in responding to the questions is provided by resting the cursor over the highlighted word in each section or by reviewing the “Comment” box in the margin of each page (Word version).

PROTOCOL SUBMISSION

Submit the completed documents electronically as an email attachment along with other required forms (e.g., hazardous substances), to the IACUC Coordinator at each institution at which any animal work will occur. Consult the Institutional Animal Care and Use Committee (IACUC) Coordinator at your institution to determine institution specific submission requirements and processing procedures. Only word processed (minimum font size of 11 point) submissions will be accepted. The “Investigator Assurance” and “Participant Qualifications” pages must be included with the submission. Final approval cannot be granted until all signed signature pages are received.

ANNUAL REVIEWS

Animal use protocols must be renewed annually using the "Annual Review – Request to Use Animals" form. Protocols continuing longer than three years must be resubmitted in their entirety using the complete “Request to Use Animals” form prior to the three year anniversary of the original protocol. Although submission timelines may vary by institution, continuing protocols must be submitted at a time sufficiently prior to the expiration date to allow adequate time for IACUC review. Animal work covered by protocols that are not approved by the IACUC prior to their expiration date will be suspended until a new protocol is approved.

MODIFICATIONS

Any proposed changes to the animal work described in an approved protocol must be reviewed and approved by the IACUC before they are initiated. Submit any proposed changes to the IACUC on the “Modification – Request to Use Animals” form.

GENERAL

The information requested on the "Request to Use Animals" and related documents is needed to enable the IACUC to fulfill its regulatory requirement to review all research, teaching, and testing activities involving live vertebrate animals. Although the information provided will be treated confidentially by each of the IACUC's to the extent permitted by law, this document may be made available to the general public in response to Ohio Open Records Act requests filed with public institutions. Responses that are both professional and comprehensible to the layperson are encouraged. Feel free to contact the IACUC Coordinator or other designated IACUC spokesperson at your institution for advice in completing the form.

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

REQUEST TO USE ANIMALS

1. PROTOCOL SUMMARY

1. A. Protocol Title:

1. B. Principal Investigator's Institution:

Kent State University

1. C. Facility(ies) where animals will be housed: *If animals will be housed in a facility not listed below, please identify the location under "OTHER LOCATION".*

UNIVERSITY OF AKRON	☐	SUMMA HEALTH SYSTEM	☐
KENT STATE UNIVERSITY		YOUNGSTOWN STATE UNIVERSITY	
Cunningham	☐	Cushwa	☐
Kent	☐	DeBartolo	☐
Tuscawaras	☐	Ward Beecher	☐
NEOMED	☐	OTHER	☒

OTHER LOCATION (Institution, building & room OR geographic location for field studies):

Ranomafana National Park, Madagascar

1. D. Source of funding for the project:

INTERNAL ☐
EXTERNAL ☒

For external awards, identify the agency(ies) and award number(s).

National Science Foundation, Biological Anthropology Program
Recommended for funding

1. E. Anticipated start date:

January, 2020

1. F. Expected animal use over the three year approval period: *Summarize all animal use by species.*

Species	Number	Source
Hapalemur aureus	10-15	Ranomafana National Park
Eulemur rufifrons	10	Ranomafana National Park

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

1. G. If this protocol is a continuation of a previously approved protocol, indicate the protocol number and provide a brief summary of the progress made to date. *Your response is limited to the space provided.* **N/A:**

Previous protocol number:

Brief summary of progress/results:

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

1. H. Project overview. *The response MUST be in lay terminology and understandable to a person with no scientific background.*

(1) Describe the medical condition, scientific question, or teaching value that is being addressed and its importance.

Cyanide is a poison known for its ubiquity among plants and low threshold for toxicity. Nonetheless, three species of bamboo lemur in and around Ranomafana National Park, Madagascar, focus most of their feeding time on various parts of Malagasy giant bamboo, which exposes the golden bamboo lemur (*Hapalemur aureus*) to 12-50 times their estimated lethal dose of cyanide on a daily basis. In addition to addressing the decades-old puzzle of how these lemurs are able to tolerate high level of this poison, this study stands to provide unique insights into the toxicology of cyanide, which has a history of application by militaries, terrorist organizations, and other malevolent entities. By elucidating how a primate such as *H. aureus* can avoid cyanide's effects, this study may inform the development of therapies, antidotes, or prophylactics to cyanide exposure in humans.

(2) List the goals of the project.

1. Create a reference-quality whole-genome assembly for the critically endangered golden bamboo lemur, *Hapalemur aureus*. This entails sequencing most of one specimen's genome to an averaging sequencing depth of 50-60 reads per site and piecing these reads together into long stretches, or scaffolds, in order to reconstruct entire chromosomes or otherwise long sections of the animal's genome.
2. Compare the gene expression profile of whole blood for *H. aureus* to that of *Eulemur rufifrons*, a close relative that does not consume such a toxic diet but lives in the same forests as the study populations of *H. aureus*.
3. Analyze the levels of cyanide, thiocyanate, and other small molecules in the blood serum of several *H. aureus* and *E. rufifrons*.

(3) Provide a chronological <u>summary</u> of the animal use from the beginning of the project through its end. *A lay description of the experimental design can be used as the response IF it addresses the intent of the question. <u>Do not</u> provide detailed descriptions of the procedures here.*

Co-PI Morgan Chaney will travel to Ranomafana National Park in Madagascar. There, he and a team of trained or licensed professionals will tranquilize a number of free-ranging *Hapalemur aureus* and *Eulemur rufifrons*and draw approximately 6 mL of blood per animal. The animals will not be removed from the exact site where they are anesthetized and will be released back into that specific microhabitat upon regaining consciousness. The blood samples will be processed immediately after harvesting and placed in frozen storage until eventual analysis back in the United States. These samples will be used to complete Goals 2 and 3.

Also while at Ranomafana, co-PI Chaney will take postmortem tissue from a previously deceased *H. aureus* that is currently in ultracold storage there. This will be used to complete Goal 1.

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

2. DESCRIPTION OF PROCEDURES INVOLVING LIVE ANIMALS

Please review all parts of this section before answering because there are separate parts for specific types of animal use. Each part will expand to accommodate the response. Mark N/A for sections that do not apply.

2. A. Animal Identification:

Indicate how animals will be identified. Multiple methods may be selected.

CAGE CARD	☐	COLLAR/TAG	☐
EAR PUNCH/NOTCH	☐	EAR TAG	☐
INDELIBLE MARKER	☐	MICROCHIP	☐
TATTOO	☐	OTHER (describe below)	☐

Describe the identification procedure if it involves penetration of the skin. Toe clipping is discouraged and, if it is used, a justification must be provided.

In order to avoid resampling individuals, a semi-permanent and non-toxic marker (e.g., dye, paint) will be applied to each animal's fur when they are sedated.

2. B. Breeding: N/A: X

Describe the breeding scheme that will be used. Indicate weaning age of offspring.

2. C. *Genotyping:* N/A:

Describe the method used to genotype the animals. Include the amount of tissue taken, age of animals, method of analgesia, and method of instrument sterilization.

To accomplish our first objective in Section 1.H.2, we will use tissue (likely spleen or liver) from a deceased infant *H. aureus* that is currently in -80°C storage at Centre ValBio. This animal died of natural causes during a previous field season. We will extract genomic DNA from this tissue, isolate high molecular weight DNA from these extracts, and submit these processed samples to an external facility for high-throughout (or "next generation") sequencing on an Illumina platform with upstream microfluidic partitioning using the 10X Chromium sequencing platform.

Our second objective in Section 1.H.2 will be accomplished by extracting total RNA from the blood samples drawn from live, free-ranging, adult *H. aureus* and *Eulemur rufifrons*. This total RNA will be shipped on dry ice to an external facility, where it will be further processed and sequenced on an Illumina platform.

2. D. Experimental manipulations: N/A:

List and describe in detail all nonsurgical experimental manipulations carried out on live animals. Euthanasia is to be described in 2.F. The response must include a statement of the known or expected impact of each procedure on animal well-being.

Groups of *H. aureus* and *Eulemur rufifrons* will be located in Ranomafana National Park using the aid of staff workers from Madagascar National Parks. Telazol (5-6 mg/kg body weight) will be injected by intramuscular injection. This will be delivered by a pneumatically fired dart fired by an experienced marksman, who will be aiming for the anterior/lateral thigh in order to target the quadriceps femoris muscle. Darted animals will be caught in stretchers after the Telazol takes effect.

While sedated, two blood samples of ≥3 mL each will be drawn from each animal into vacuum tubes by the co-PI, an experienced veterinarian, and his/her assistant. Opportunistic measurements will also be

taken of the animal's weight, as well as other non-invasive metrics (e.g., anatomical length measurements). The animal will be treated with an antiparasitic medicine. As mentioned herein, the animals' vital sign will be monitored continually and they will be marked by a semi-permanent marker in order to prevent resampling from the same animal at a later time.

2. E Surgical manipulations. N/A: X

2.E.(1) Description of surgical procedures:

Describe each surgical procedure under a separate heading. Procedures that are performed on the same animal at the same time may be described as one procedure. IF more than four different surgeries are planned, then similar ones may be combined into a single response.

Surgical procedure #1:

Is the surgical procedure a survival procedure? **Yes:** **No:**

Describe the procedure in detail. Include the pre-operative preparation of the animal, a description of the aseptic technique and how instruments and implantable devices are sterilized. The response must include a statement of the known or expected impact of the procedure on animal well-being.

Surgical procedure #2:

Is the surgical procedure a survival procedure? **Yes:** **No:**

Description of procedure (instructions as above):

Surgical procedure #3:

Is the surgical procedure a survival procedure? **Yes:** **No:**

Description of procedure (instructions as above):

Surgical procedure #4:

Is the surgical procedure a survival procedure? **Yes:** **No:**

Description of procedure (instructions as above):

2.E.(2) Multiple major survival surgery:

Does this project involve multiple major survival surgeries in the same animal? **YES:**
NO:

If so, provide a justification.

2. F. Anesthesia/Sedation. N/A:

List the procedures that require anesthesia or sedation individually below and describe the anesthetic regimen used for each. If multiple procedures use the same anesthetic regimen, then they can be combined into one response.

Anesthesia/sedation procedure #1:

Identify the procedure requiring anesthesia or sedation. List all drugs (including neuromuscular blocking agents) used as part of the anesthetic/sedative regimen; include the dose, route of administration and indicate the frequency of repeat dosing. If animals will be anesthetized with inhalants, indicate the percentage of anesthetic gas, any auxiliary gases used, oxygen flow rate and ventilatory parameters (for mechanically ventilated animals).

Telazol (5-6 mg/kg body weight) will be injected by intramuscular injection. This will be delivered by a pneumatically fired dart.

Describe the procedures and equipment used to monitor the depth of anesthesia and animal well-being. If neuromuscular blocking agents are used, include techniques that are reliable in paralyzed animals.

During the procedure, the individuals will be monitored once every five minutes. A veterinarian will be present to monitor the following clinical parameters: heart rate, appearance, respiratory rate, and body temperature.

Describe the supportive measures to assure animal well-being while under anesthesia.

The potential adverse effects of administering Telazol are excessive salivation and drying of the eyes. In order to counter the excessive salivation, atropine (.54 mg/kg body weight) can be administered, and to counter any desiccation of the eyes an ophthalmic ointment will be applied. While under anesthesia, it is always a possibility that larger complications can occur such as depressed breathing or depressed heart rate, but these can be countered by monitoring vital rates (see above). All procedures will be conducted with a veterinarian present.

The potential adverse effect of drawing blood is that there is the potential for infection at the site of venipuncture. A topical antibiotic solution will be applied to the site of the blood removal to prevent possible infections.

The only other major risk posed by all other procedures is stress due to handling, which will be minimized by gentle handling, the wearing of PPE, close monitoring of each individual, and the ability to inject additional Telazol if an animal shows signs of waking up. The animal will be expected to wake up from the Telazol after 30-45 minutes of sedation.

Anesthesia/sedation procedure #2:

Identify the procedure requiring anesthesia/sedation and describe the anesthetic regimen as indicated above.

Procedures and equipment used to monitor the depth of anesthesia and animal well-being:

Supportive measures:

Anesthesia/sedation procedure #3:
Identify the procedure requiring anesthesia/sedation and describe the anesthetic regimen as indicated above.

Procedures and equipment used to monitor the depth of anesthesia and animal well-being:

Supportive measures:

Anesthesia/sedation procedure #4:
Identify the procedure requiring anesthesia/sedation and describe the anesthetic regimen as indicated above.

Procedures and equipment used to monitor the depth of anesthesia and animal well-being:

Supportive measures:

2. G. Building(s) and room number(s) where the procedures will take place:

Nonsurgical Procedures:

Surgical Procedures:

2. H. Postprocedural care and monitoring:

1) Describe the post-procedural care and monitoring for both surgical (after recovery from anesthesia) and nonsurgical procedures. Identify the parameters being monitored and the frequency and duration of monitoring for each study related procedure. Include how records of the care will be maintained and their location.

The animals will be monitored during recovery from the Telazol at the location where they were sedated until they awake. During the transitional period between sedation and full consciousness, the lemurs will be gently handled to reduce stress and given water to prevent dehydration. As mentioned in Section 2F, the animal may be given small doses of atropine and ophthalmic ointment to counteract possible negative side effects of the Telazol. Everything will be done in the presence of a veterinarian.

2) Identify by title who will conduct the care and monitoring.

KSU graduate student
Executive Director of Centre ValBio (30 years' experience in similar methods of sedation)
Malagasy veterinarian and his/her assistant

*3) List any analgesics or other medically related pharmaceutical agents that animals may receive. Include **a)** dose, **b)** route of administration **c)** frequency of administration, and **d)** duration of therapy.*

Ivermectin (dose of 0.2 mg/kg BW), delivered once by subcutaneous injection

4) List the criteria that will be used to determine that relief from pain or distress is needed and how the adequacy of that relief will be assessed.

Because the lemurs will experience momentary and minimal pain during this procedure, analgesia is not necessary.

5) List the humane endpoints that will be used to euthanize an animal or otherwise remove an animal from a study.

N/A

2. I. Disposition of animals:

Describe the method of euthanasia including the name, dose, and route of administration of any pharmaceutical agents used. Describe the method(s) that will be used to confirm death. Animals euthanized by an overdose of carbon dioxide must undergo a secondary method of euthanasia to confirm death. If animals will not be euthanized, describe their disposition.

N/A

2.J. Chemical/compound administration to live animals

Are all of the chemicals (e.g., test compounds, receptor agonists/antagonists, labeling compounds, anesthetics, analgesics, euthanasia agents, etc.) administered to live animals commercially available pharmaceutical preparations intended for animal or human use?

Yes: X **No:**

If not, then complete the following for each product.
Identify the chemical/compound and describe how it is prepared and stored to assure appropriate purity, sterility and suitability for administration to animals. Indicate the shelf life of the prepared product.

Are all of the chemicals/compounds listed above pharmaceutical grade? **Yes: X** **No:**

If not, then list them and provide a justification for not using a pharmaceutical grade preparation.

3. SPECIAL CONSIDERATIONS

Mark N/A for sections that do not apply.

3.A. Food/ fluid restriction: N/A: X

*If the study involves scheduling access to food or fluid OR restricting food or fluid intake beyond that associated with a routine overnight pre-procedural fast or weight control, then describe **a)** the amount and time of the restriction, **b)** expected impact on animal well-being, and **c)** criteria for removal of the restriction.*

Describe the record-keeping associated with ongoing restrictions. Indicate where the records will be maintained. At a minimum animal weights must be documented once weekly and food/water consumption noted daily.

3. B. Prolonged restraint: N/A: X

*If the project involves more than routine restraint of conscious animals for brief periods, then describe: **a)** the restraint, **b)** its duration and frequency, **c)** how animals will be conditioned to it, and **d)** how frequently animals will be observed while restrained.*

Provide a justification for the restraint.

3. C. Immunologic adjuvants: N/A: X

If the project involves the use of immunologic adjuvants (e.g., Freund's adjuvant, RIBI adjuvant) complete the following.

	First Injection	*Second Injection*	*Subsequent Injections*
Adjuvant			
Anatomic site of injection & route			
Number of sites			

Volume per site			
Time interval between injections			

3. D. Dog exercise: N/A: X

If the project involves the use of dogs, indicate if any animals will be exempted from the dog exercise program and include the duration of the exemption and a justification for it. If there are no exemptions, enter "no exemptions".

3. E. Environmental enrichment for primates: N/A: X

If the project involves the use of nonhuman primates, indicate if any animals will be exempted from the environmental enrichment program for primates and include the duration of the exemption and a justification for it. If there are no exemptions, enter "no exemptions".

3. F. Housing or enrichment restrictions: N/A: X

If the project involves the single housing of animals of a social species OR exemption from normal environmental enrichment, then describe and provide a justification for the restriction.

3. G. Hazardous material use: N/A: X

If the project involves the administration of any potentially hazardous materials to live animals, complete the following for each material and attach the appropriate hazardous material form(s) required by the institution at which the work will take place.

Name of hazardous agent(s):

Select the appropriate classification of hazard(s)

CARCINOGEN	☐	INFECTIOUS AGENT	☐
RADIOACTIVE ISOTOPE	☐	RECOMBINANT NUCLEIC ACID	☐
TOXIN	☐	HUMAN TISSUE/CELLS	☐
OTHER	☐		

Describe the potential health effects of the hazard and list the possible routes of exposure hazard:

Number of animals receiving material:

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

3. H. Genetically modified animals: N/A: X

If the project involves the use, breeding, or creation of genetically modified animals, complete the following for each genotype.

List the animals by genotype and describe the known or expected impact of the associated phenotype on animal well-being:

Describe the measures to relieve or manage pain or distress related to each phenotype that is associated with an adverse impact on animal well-being:

Will any new genetically modified animals be created in the project? **Yes:** **No:**

If so, describe the monitoring associated with the new line to assure adequate provision of humane animal care. Previously undescribed phenotypic conditions that negatively impact animal well-being must be reported to the IACUC:

3.I. Animal housing outside of main animal facility: N/A: X

If animals will be maintained outside of the main animal facility longer than 12 hours for USDA covered species or longer than 24 hours for all others, then complete the following.

Identify the building, room number, species, and number of animals to be housed. Indicate the duration of housing.

Provide a justification for the extramural housing.

Has the IACUC previously approved the location? **YES:** **NO:**

3.J. Field studies: N/A:

If the project involves the use of animals in a field setting, complete the following.

Identify the occupational health and safety issues associated with studying the species in the wild.

Lemurs are primates, and as such there is some potential for zoonotic disease. This will be lessened through the use of protective equipment such as nitrile gloves and surgical masks. In addition to routine immunizations, the KSU graduate student performing the fieldwork will be additionally immunized against hepatitis A, hepatitis B, typhoid, yellow fever, rabies, and polio; this variety of immunizations is aligned with CDC recommendations for Americans traveling to Madagascar.

Describe the potential impact of the study on native populations of the species being studied and others that may be affected by the study.

There will be a minimal or negligible impact on native populations because the anesthetized animals will regain consciousness and be released in the same habitat where they were initially sedated.

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

List and attach the permits and other necessary permission documents that are needed to carry out the study.

Attached is a letter of permission and endorsement from the Executive Director of Centre ValBio, the field station in Ranomafana where this study will be conducted.

3.K. Procedures performed at a supplier location: N/A: X

If animals will undergo experimental or surgical procedures at a supplier's location, complete the following and attach a statement from the supplier confirming IACUC approval of the procedure. Identify the procedure and supplier's Public Health Service Animal Welfare Assurance number and USDA registration number (as applicable).

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

4. CLASSIFICATION OF PROCEDURES ACCORDING TO LEVEL OF PAIN AND/OR DISTRESS

Mark the appropriate category for each animal procedure and identify the procedure(s) in the spaces provided. List the number of animals in each pain category in the box provided. If individual animals will undergo procedures in multiple pain categories, then include them in the tabulation for the highest pain category.

☒ - **Category C** - Procedures that involve no more than momentary or slight pain or distress.

List procedures:

Animals will be located in the forest with the help of a trained guide from Madagascar National Parks, and a stretcher will be suspended directly below the animal's position in the forest canopy. The animal will be darted with a sedative (Telazol; see below) and caught in the stretcher. At this point, blood samples and measurements will be taken while the animal is unconscious. The animals will not be moved from the site where they are darted, and they will be released back into this habitat.

Number of animals in category C:

10-15 Hapalemur aureus and up to 10 Eulemur rufifrons

☐ - **Category D** - Procedures that may cause more than momentary or slight pain or distress for which appropriate analgesia, anesthesia or tranquilization is provided.

List procedures:

Number of animals in category D:

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

☐ - **Category E** - Procedures that may cause pain or distress which are not relieved by analgesia, anesthesia, or tranquilization.
List procedures:

Number of animals in category E:

For Category E procedures: Provide a detailed scientific justification for withholding analgesia, anesthesia, and tranquilization.

5. ALTERNATIVES TO THE USE OF ANIMALS AND PAIN OR DISTRESS PRODUCING PROCEDURES

Provide a written narrative description of the methods and sources that were used to determine that suitable alternatives to the use of animals and to the pain or distress producing procedures described in the protocol are not available. Provide an explanation for alternatives that were identified but deemed unsuitable. Literature searches must include a) databases searched, b) the date of the search, c) the years covered by the search (minimum 10 years), and d) the search strategy including keywords used. At least two acceptable information sources must be used. The response must address the three R's: Replacement models, Refinements in technique, and Reduction in animal numbers. Information sources that are commonly used include http://www.pubmed.gov, http://agricola.nal.usda.gov, http://www.nal.usda.gov/awic, and specifically for teaching activities, http://oslovet.veths.no.

We queried NCBI's PubMed database (on June 22, 2019) and Clarivate Analytics's Web of Science database (on July 3, 2019) using the following key terms: cyanide intoxication, animal model, cyanide detection, non-invasive, and RNA sequencing sample size

A recent replacement animal model is a line of mice bred by Sabourin et al. (2016: *Int J Toxicol* 35*(5)*). The acute oral lethal dose (as LD_{50}) in this line of rodent was between 9.9 and 11.8 mg of potassium cyanide per kg body weight. Such doses are very minimal in comparison with the 1850 µmol of HCN consumed by *H. aureus* (Ballhorn et al., 2009: *Am J Primatol* 71), which is equivalent to about 190 mg/kg in this species. Thus, no replacement animal model would be suitable for this study.

Most non-invasive methods for detecting cyanide exposure involve assaying the urine (e.g., Vaz et al., 2012: *PLoS One* 7*(4)*), but such studies have already been conducted on this population by others (Yamashita et al., 2010: *Am J Primatol* 72; M.E. Lauterbur, unpublished). The previous detection of such high levels of cyanide and thiocyanate in these lemurs' urine justifies follow-up work testing their blood for these compounds (as well as novel gene-expression profiles) because of the intensity of the levels

documented by previous authors.

There is currently no biological consensus on how many individual samples would be sufficient to control for false discovery in RNA-sequencing experiments, but it is uncontroversial that the adding of more individuals (or biological replicates) will improve statistical power (Liu et al., 2014: *Bioinformatics* 30*(3)*). Although some comparative RNA-sequencing work has used as few as four individuals per species (Perry et al., 2012: *Genome Res* 22*(4)*), we aim to maximize statistical power in our analysis. Furthermore, because it is likely that multiple *H. aureus* populations within Ranomafana National Park will be sampled as part of this protocol, we see the potential for a second use of these samples in documenting the population genetics of this critically endangered species as part of a future study.

6. JUSTIFICATION FOR THE USE OF ANIMALS

6. A. Provide a rationale for involving animals.

The bloodstream of these animals was identified as a tissue of special interest for this study because it plays an important role in the toxicodynamics of cyanide poisoning. In this case, the animal is known to consume cyanogenic foods nearly exclusively for a large part of the year and its behavioral response shows no adverse effects. Thus, it is warranted to directly measure levels of cyanide, its byproducts, and detoxification substrates in the blood.

6. B. What is the basis for selecting the species that you have chosen?

No other species is known to be able to tolerate such high doses of cyanide, with the possible exception of the closely related greater bamboo lemur (*Prolemur simus*).

6. C. Number of animals requested:

Provide a justification for the number of animals requested. Identify the species, genotypes, strains, and/or stocks of animals. Include other descriptors as relevant (e.g., age or weight, gender, timed pregnant). For research protocols, list the experimental and control groups and indicate the number of animals in each. Include the statistical justification, or other basis, for selecting the number requested. If a research protocol includes the use of animals solely for training (i.e., the training does not occur as part of the experimental use of animals), then include the expected number of animals to be used for training. Animals used for training can be justified by documenting the expected number of persons to be trained and the number that can be trained per animal.

We hope to sample at least two *H. aureus* populations within Ranomafana National Park, and part of this study includes RNA sequencing experiments to detect differentially expressed (DE) genes. Previous work (Williams et al., 2014, *Curr Protocols Hum Genet* 38*(1)*) recommends sequencing at least three biological replicates per population, assuming 30 million reads per sample, in order to detect DE genes between populations. Other work (Liu et al., 2014: *Bioinformatics* 30*(3)*) rounds out this recommendation by showing that the number of DE genes increases with the addition of up to four more replications (i.e., seven in the study by Liu et al., 2014). The statistical power, with an FDR of 0.05 and 30 million reads per sample, roughly converges in experiments with more than 5 replicates (Liu et al., 2014). Thus, we have decided 10-15 animals between two populations would be sufficient for our purposes.

6. D. Provide written assurance that the use of animals described in this protocol does not unnecessarily duplicate previous experiments.

A previously published study measured cyanide or its metabolite thiocyanate in the urine (Yamashita et al., 2010, *Am. J. Primatol.* 72); and more recent, but unpublished work by a collaborator (M. Elise Lauterbur, Stony Brook U.) has measured the precise levels of these compounds in the urine using mass-spectrometric methods. No work up to this point, however, has attempted to measure these compounds in the bloodstream of these animals.

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

7. HOUSING AND HUSBANDRY

7. A. Indicate the approximate number of animals to be housed at one time and approximate duration of housing.

None. No animals will be housed because they will be only briefly sedated before being released back into their original habitats.

7. B. If rodents are to be housed, is there a preference as to the type of caging (i.e., plastic, wire-bottom, microisolator or other) OR the number of animals per cage? **YES: NO:**

If yes, please specify. Note that the use of wire-bottom cages or single housing of animals requires a justification.

N/A

7. C. Will a light cycle other than the standard 12 hours light/12 hours dark be necessary for any of the animals on this protocol? **YES: NO: X**

If yes, please specify the light cycle(s) and indicate the group(s) of animals that will require it.

7. D. Will the animals on this protocol have any special temperature or humidity requirements? **YES: NO: X**

If yes, please describe.

7. E. Will the animals on this protocol require a special diet or special water? **YES: NO: X**

If yes, please identify the product, the number of animals receiving it, and who will prepare and administer it.

7. F. Will the animals on this protocol require any other special housing, care, environmental conditions, or other considerations? **YES: NO: X**

If yes, please describe.

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

7. G. Will it be necessary to house animals after they have received any hazardous materials (refer to Part 3.G.)? **YES:** **NO: X**

If yes, please identify the material, the number of animals, and the duration of housing. Describe how the housing cages and room will be identified to alert personnel that a hazard is present.

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

8. PROTOCOL APPROVAL

Click "Choose Institution" to select the institution to which the protocol will be submitted.

Protocol approval is indicated by the signatures of the institution-specific individuals identified below. The individuals signing confirm that they have reviewed the protocol and find it to be in compliance with applicable animal care and use regulations and institutional policies.

Kent State University

Approval Signatures:

_____ Date _____
Facility Director

_____ Date _____
Department Chair/Research Director

_____ Date _____
IACUC Member

_____ Date _____
Attending Veterinarian

_____ Date _____
IACUC Chairperson

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

INVESTIGATOR ASSURANCE

By signing below I/we agree to:

A. Employ procedures that will avoid or minimize discomfort, distress, and pain to animals, consistent with sound research design.

B. Comply with the protocol as approved by the Institutional Animal Care and Use Committee (IACUC) and to obtain the consent of the IACUC before implementing any changes to the protocol.

C. Comply with the policies of the IACUC of the institution at which this work is conducted, the National Research Council Guide for the Care and Use of Laboratory Animals, the Public Health Service Policy on Humane Care and Use of Laboratory Animals, the regulations of the Animal Welfare Act and other applicable federal, state and local regulations governing the use of animals in research, teaching, and testing.

D. Maintain adequate records of all animal experimentation procedures.

E. The provision of emergency veterinary care including euthanasia by the attending veterinarian or his/her designee for animals showing evidence of unbearable pain, distress, or illness with the understanding that an effort will be made to contact me or my designee prior to the initiation of any treatment.

Principal Investigator:

Name: Anthony Tosi Department: Anthropology

Email address: atosi@kent.edu Telephone number: [redacted]

Signature _____ Date: _____

Co-Investigator:

Name: Morgan Chaney Department: Anthropology

Email address: mchaney1@kent.ed Telephone number: [redacted]

Signature _____ Date: _____

Co-Investigator:

Name: Department:

Email address: Telephone number:

Signature _____ Date: _____

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

PARTICIPANT QUALIFICATIONS

Complete this form for the principal investigator, each co-investigator, and each of the individuals who may participate in the animal work described in the protocol. By signing below the participant acknowledges that he/she has read the protocol and agrees to comply with it.

NAME: Dr. Anthony J. Tosi, Ph.D.

TITLE: Associate Professor

EMAIL: atosi@kent.edu

List the participant's responsibilities on the protocol.

PI Tosi will not participate in the field component of this protocol. He will provide oversight and guidance during the phase of the research following co-PI Morgan Chaney's return from the field.

Describe the participant's experience and/or qualifications relevant to the responsibilities on the protocol. If the participant has no relevant experience then check here ☒ and identify below who will be responsible for training.

EXPERIENCE/QUALIFICATIONS:

Dr. Patricia C. Wright will provide training for co-PI Chaney.

DESCRIPTION OF FORMAL ANIMAL CARE AND USE TRAINING:

TITLE OR DESCRIPTION OF TRAINING	LOCATION	DATE OF TRAINING

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

PARTICIPANT SIGNATURE DATE

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

PARTICIPANT QUALIFICATIONS

Complete this form for the principal investigator, each co-investigator, and each of the individuals who may participate in the animal work described in the protocol. By signing below the participant acknowledges that he/she has read the protocol and agrees to comply with it.

NAME: Morgan E. Chaney

TITLE: Ph.D. Candidate

EMAIL: mchaney1@kent.edu

List the participant's responsibilities on the protocol.

Co-PI Chaney will assist the veterinarian and Madagascar National Parks staff in the sedation of the lemurs and the collection of blood samples.

Describe the participant's experience and/or qualifications relevant to the responsibilities on the protocol. If the participant has no relevant experience then check here ☒ and identify below who will be responsible for training.

EXPERIENCE/QUALIFICATIONS:

Dr. Patricia C. Wright will provide training for co-PI Chaney.

DESCRIPTION OF FORMAL ANIMAL CARE AND USE TRAINING:

TITLE OR DESCRIPTION OF TRAINING	LOCATION	DATE OF TRAINING
CITI Basic Biosafety Training	http://www.citiprogram.org/	14-Feb-2019
CITI Animal Biosafety	http://www.citiprogram.org/	21-June-2019
Responsible Conduct of Research (BMS 61000)	Northeast Ohio Medical University	Fall, 2015

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

PARTICIPANT SIGNATURE DATE

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

PARTICIPANT QUALIFICATIONS

Complete this form for the principal investigator, each co-investigator, and each of the individuals who may participate in the animal work described in the protocol. By signing below the participant acknowledges that he/she has read the protocol and agrees to comply with it.

NAME: Dr. Patricia C. Wright, Ph.D.

TITLE: Executive Director and Founder of Centre ValBio

EMAIL: [redacted]

List the participant's responsibilities on the protocol.

Supervision of darting and sample collection during experimental procedures. Dr. Wright will also train co-PI Chaney while he is visiting Centre ValBio.

Describe the participant's experience and/or qualifications relevant to the responsibilities on the protocol. If the participant has no relevant experience then check here ☐ and identify below who will be responsible for training.

EXPERIENCE/QUALIFICATIONS:

Dr. Wright has 30 years of experience in collecting measurements from lemurs in Ranomafana National Park following protocols using sedative darts and similar methods outlined here.

DESCRIPTION OF FORMAL ANIMAL CARE AND USE TRAINING:

TITLE OR DESCRIPTION OF TRAINING	LOCATION	DATE OF TRAINING
Workshops on the sedation and capture of lemurs, trained by Dr. Kenneth Glander, Ph.D. and several DVMs	Duke University	1987-1990
Hands-on training in the sedation and capture of lemurs, trained by Dr. Kenneth Glander, Ph.D. and several DVMs	Ranomafana National Park, Madagascar	1987-1990

PARTICIPANT SIGNATURE _____ DATE _____

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

PARTICIPANT QUALIFICATIONS

Complete this form for the principal investigator, each co-investigator, and each of the individuals who may participate in the animal work described in the protocol. By signing below the participant acknowledges that he/she has read the protocol and agrees to comply with it.

NAME:

TITLE:

EMAIL:

List the participant's responsibilities on the protocol.

Describe the participant's experience and/or qualifications relevant to the responsibilities on the protocol. If the participant has no relevant experience then check here ☐ and identify below who will be responsible for training.

EXPERIENCE/QUALIFICATIONS:

DESCRIPTION OF FORMAL ANIMAL CARE AND USE TRAINING:

TITLE OR DESCRIPTION OF TRAINING	LOCATION	DATE OF TRAINING

PARTICIPANT SIGNATURE _____ DATE _____

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

PARTICIPANT QUALIFICATIONS

Complete this form for the principal investigator, each co-investigator, and each of the individuals who may participate in the animal work described in the protocol. By signing below the participant acknowledges that he/she has read the protocol and agrees to comply with it.

NAME:

TITLE:

EMAIL:

List the participant's responsibilities on the protocol.

Describe the participant's experience and/or qualifications relevant to the responsibilities on the protocol. If the participant has no relevant experience then check here ☐ and identify below who will be responsible for training.

EXPERIENCE/QUALIFICATIONS:

DESCRIPTION OF FORMAL ANIMAL CARE AND USE TRAINING:

TITLE OR DESCRIPTION OF TRAINING	LOCATION	DATE OF TRAINING

PARTICIPANT SIGNATURE _____ DATE _____

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

PARTICIPANT QUALIFICATIONS

Complete this form for the principal investigator, each co-investigator, and each of the individuals who may participate in the animal work described in the protocol. By signing below the participant acknowledges that he/she has read the protocol and agrees to comply with it.

NAME:

TITLE:

EMAIL:

List the participant's responsibilities on the protocol.

Describe the participant's experience and/or qualifications relevant to the responsibilities on the protocol. If the participant has no relevant experience then check here ☐ and identify below who will be responsible for training.

EXPERIENCE/QUALIFICATIONS:

DESCRIPTION OF FORMAL ANIMAL CARE AND USE TRAINING:

TITLE OR DESCRIPTION OF TRAINING	LOCATION	DATE OF TRAINING

For IACUC Use Only		
Category:	Special Considerations:	Protocol #:

PARTICIPANT SIGNATURE DATE

REPOBLIKAN'I MADAGASIKARA
Fitiavana - Tanindrazana - Fandrosoana

SECRETARIAT GENERAL

DIRECTION GENERALE DE L'ENVIRONNEMENT ET DES FORETS

DIRECTION DE LA GESTION DES RESSOURCES NATURELLES RENOUVELABLES ET DES ECOSYSTEMES

AUTORISATION DE RECHERCHE

N°002 20/MEDD/SG/DGEF/DGRNE

NOM ET PRENOM	Morgan E. Chaney
FONCTION	Chercheur
ACCOMPAGNE DE	Un vétérinaire, un étudiant MZBA, un représentant CAFF/CORE
ORGANISME TUTEL	Mention Zoologie et Biodiversité Animale (MZBA) – BP 906- Antananarivo
LIEU	PN Ranomafana
DUREE	Trois (03) mois à partir de Janvier 2020

EST AUTORISE (E) A FAIRE DES RECHERCHES SUR :

« Recherche d'adaptation génomiques et d'expressions pour désintoxication au cyanure chez *Hapalemur aureus* »

MENTION SPECIALE D'ACTIVITES

Espèces cibles : *Hapalemur aureus* et *Eulemur fulvus*

-Capture avec relâche à l'aide de fléchettes contenant du Tetazol
-Collecte d'échantillons de sang sur les individus capturés (3ml)
-Extraction d'ARN pour des analyses génétiques

AUCUN DEVELOPPEMENT DE PRODUITS N'EST AUTORISE

EXPORTATION : Quatre (04) échantillons de sang par espèce par quatre (04) sites

OBLIGATIONS DU TITULAIRE :

- Négocier avec les gestionnaires et/ou comité de gestion des sites ou forêts transférées pour y accéder, le cas échéant
- **Effectuer une restitution au niveau Régional avec le procès-verbal y afférent signé par le DREDD ou son représentant**
- Faire viser la présente par la Direction Régionale de l'Environnement et du Développement Durable et/ou DREDD concernées avant toute descente sur terrain conformément à la note **n° 394-10/MEF/SG/DGF/DVRN/SGFF du 18 Mai 2010** de la localité de recherche
- Remettre à la Direction de la Gestion des Ressources Naturelles renouvelables et des Ecosystèmes, en quatre (04) exemplaires EN FRANÇAIS, le rapport préliminaire à la fin de sa mission et le rapport final avec les résultats des recherches au plus tard UN an après la mission, en versions papier et électronique.
- **Respecter la réglementation en matière forestière et toute irrégularité aux mentions de l'Autorisation de Recherche est considéré comme un délit forestier**
- Pour tout transport de produits de collecte (faune et flore), avoir un procès-verbal de constatation des collectes effectuées par le CEDD concerné et autorisation de transport délivré par DREDD si le déplacement se fait en dehors de la région et remettre une copie au DGRNE
- Pour toute exportation : remettre une copie du dépôt au DGRNE et une autre au dossier d'exportation

AMPLIATIONS :

CAFF/CORE
DREDD : Vatovavy Fitovinany
CEDD : concernées
Communes concernées
« Pour contrôle et suivi »
DGEF
« Pour contrôle et suivi »
MZBA
« Pour le rapport »

Antananarivo, le 09 JAN 2020

LE DIRECTEUR DE LA GESTION DES RESSOURCES NATURELLES RENOUVELABLES ET DES ECOSYSTEMES

pi

MINISTERE DE L'ENVIRONNEMENT ET DU DEVELOPPEMENT DURABLE
DIRECTION GENERALE DE L'ENVIRONNEMENT ET DES FORETS
DIRECTION DE LA GESTION DES RESSOURCES NATURELLES RENOUVELABLES ET DES ECOSYSTEMES

Re: [EXTERNAL] Re: CITES Permit App 56547D

Cate, Emily B <emily_cate@fws.gov>

Tue 4/21/2020 8:43 AM

To: Elise Lauterbur <lauterbur@gmail.com>

Hi Elise,

Yes, that is correct. Thank you for letting me know. I will move forward with processing this application. Please let me know if you have any questions or concerns.

Regards,
Emily

From: Elise Lauterbur <lauterbur@gmail.com>
Sent: Monday, April 20, 2020 1:24 PM
To: Cate, Emily B <emily_cate@fws.gov>
Subject: Re: [EXTERNAL] Re: CITES Permit App 56547D

Good morning Emily,

I hope you had a good weekend! Thanks so much for that information. In that case, since the approval process is the same (if it's safe to assume that the likelihood of approval is the same as well?), we'd like to set this up as a multi-use permit.

Thanks again,
Elise

On Wed, Apr 15, 2020 at 10:47 AM Cate, Emily B <emily_cate@fws.gov> wrote:

> Hi Elise,
>
> We can set this up as a multi-use import permit. It would go through the same approval process, but the final permit itself would contain the following language: "Permit may be copied for multiple shipments; permittee to retain original". Please let me know how you would like to proceed after conferring with Dr. Wright. I appreciate your time.
>
> Regards,
> Emily
>
> ---
>
> **From:** Elise Lauterbur <lauterbur@gmail.com>
> **Sent:** Wednesday, April 15, 2020 1:15 PM
> **To:** Cate, Emily B <emily_cate@fws.gov>
> **Subject:** Re: [EXTERNAL] Re: CITES Permit App 56547D
>
> Hi Emily,

It's my understanding that each permit is one use, so we had planned for a single import and just wait to do that until the rest of the samples have been collected. Is it possible to do multiple imports on a single permit? If so, I'll discuss it with Dr. Wright.

Thanks,
Elise

On Wed, Apr 15, 2020 at 10:11 AM Cate, Emily B <emily_cate@fws.gov> wrote:

> Hi Elise,
>
> Are the samples proposed to be imported through a single import or multiple imports since some of the samples have not yet been collected?
>
> Thank you,
> Emily
>
> **Emily Cate** | Permits Biologist
> U.S. Fish and Wildlife Service | International Affairs
> Division of Management Authority | Branch of Permits
> 5725 Leesburg Pike, MS:IA
> Falls Church, VA 22041-3803
>
> 
>
>
> ---
>
> **From:** Elise Lauterbur <lauterbur@gmail.com>
> **Sent:** Wednesday, April 8, 2020 4:06 PM
> **To:** Cate, Emily B <emily_cate@fws.gov>
> **Subject:** Re: [EXTERNAL] Re: CITES Permit App 56547D
>
> Hi Emily,
>
> Great, thank you. I have attached a document answering your questions called "CITES permit app 56547D - April 2020.docx," two additional spreadsheets for questions 2 and 3, and two additional files (updated IACUC and a research permit that was granted after our initial submission). I've included an additional statement on how these activities will benefit the wild *Eulemur rufifrons* population, as well as why *E. rufifrons* samples will benefit *H. aureus* and *P. simus* populations - please let me know if you need more detail.
>
> Thanks again!
> Elise
>
> On Tue, Apr 7, 2020 at 10:37 AM Cate, Emily B <emily_cate@fws.gov> wrote:
>
> > Hi Elise,

Thank you for gathering up this information. I would prefer to have it as one row per animal sampled (multiple samples per animal okay) Please also include a count of the total number of animals and let me know if you have any questions.

Regards,
Emily

From: Elise Lauterbur lauterbur@gmail.com>
Sent: Monday, April 6, 2020 5:48 PM
To: Cate, Emily B <emily_cate@fws.gov>
Subject: [EXTERNAL] Re: CITES Permit App 56547D

Dear Emily,

Thank you for your email, and for working on processing our application. I'm working on the answers to your questions - for the second question (updated table of samples to reflect January and planned February/March sampling), would you like this as a table with one row per animal sampled (in which case parts c and f would include multiple samples, eg. c. 2 blood and 1 skin biopsy samples, f. 1 9ml vial with blood in buffer, 1 2ml vial with blood, 1 vial with skin biopsy), or as previously submitted with one row per specimen type?

Thank you,
Elise

On Mon, Mar 30, 2020 at 7:33 AM Cate, Emily B <emily_cate@fws.gov> wrote:

> Dear Ms. Lauterbur,
>
> I have the application dated 09/26/2019, received 10/02/2019, regarding the proposed import of scientific samples collected from multiple species of lemurs in Madagascar. I apologize for the delay in processing your application.
>
> Please provide the following information so that I may continue to process your application:
>
> 1. Provide the name and address of the exporter in the foreign country. (Name, Address, City, State/Province, Country, Postal Code)
>
> 2. Generally, did the proposed sampling during January 2020, as described in the application, occur? If so, please provide an updated answer for the following question:
>
> For **each** animal/specimen involved in the proposed activity provide: a) Scientific Name, b) Common name, c) Number and type of sample/part, d) Wild or captive born, E) approximate date of collection (mm/yyyy), f) Description of packaging (e.g., vials, slides, envelopes, etc.), and g) Total number of all samples in the shipment.
>
> 3. If the proposed January 2020 sampling occurred, please provide an updated answer to the following if any differences occurred than what was originally submitted in the application:
>
> For **each** animal/specimen involved in the proposed activity provide: a) scientific name and common name, b) specific location of where, when, and by whom (name and address) the specimen was removed from the wild, c) Purpose of removal and length or approximate length

of time held in captivity. Discuss issues such as the method of collection, was the collection done as part of a larger study, were animals returned to the wild after sampling, and did any mortalities or injuries occur due to collection or holding, d) If and how any remuneration, either financial or in-kind, was provided for taking or capturing animals or for the collection of samples, e) Your efforts to use captive specimens (e.g., captive-born, captive-held), or parts thereof, in lieu of taking animals from the wild, f) Copies of your foreign or domestic collecting permit, license, contract, or agreement, g) Documentation showing that the specimen(s) was/were legally obtained by the applicant, and h) Copies of any applicable State, Tribal, Federal, or Foreign government permits or licenses that authorized the removal of this animal from the wild.

4. Please clarify the proposed necessary use of red-fronted brown lemurs (*Eulemur rufus*) in the study. The doctoral dissertation submitted with the application states that captive-held ring-tailed lemurs (*Lemur catta*) will be used and the corresponding IACUC submitted did not include the use of red-fronted brown lemurs. In addition, the answer to question 10(a) made reference to the brown lemur (*Eulemur fulvus*), while a reference to the red-fronted brown lemur was absent. Please clarify if there are samples proposed to be imported from the brown lemur as well. **Please note that for each species, we will need a statement on how the activities will enhance or benefit the wild population** (I see this answered for only the greater bamboo lemur (*Prolemur simus*) and the golden bamboo lemur (*Hapalemur aureus*)). If you have questions about this requirement, please let me know, as it may be easier to move forward by having separate applications for the species.

Please submit the requested information in one submission directly to me via email (attachments such as spreadsheets describing the multiple samples are encouraged). Please let me know if you have any questions or concerns.

In accordance with 50 CFR 13.11(e), if the requested information is not received by this office by **May 14, 2020**, your application will be abandoned and administratively closed. Once a file is closed you will need to submit a new application and all required fees for the Service to consider your proposed activity. Please refer to permit number 56547D in your correspondence.

Respectfully,
Emily

Emily Cate | Permits Biologist
U.S. Fish and Wildlife Service | International Affairs
Division of Management Authority | Branch of Permits
5725 Leesburg Pike, MS:IA
Falls Church, VA 22041-3803