IPS - Research Grant Application

Please complete the following application form. Do not exceed the space provided. Font should be no less than 12pt. Please direct any questions regarding your application or this award to Prof Marina Cords (marina.cords@columbia.edu).

Application Due: March 1st

Last name: LANGGENG **First name: ABDULLAH**

Title of Proposed Project: INVESTIGATING THE EFFECTS OF JAPANESE MACAQUE HOT SPRING BATHING BEHAVIOR ON PARASITISM AND GUT MICROBIOME

Affiliation (include the country): KYOTO UNIVERSITY PRIMATE RESEARCH INSTITUTE (JAPAN)

Email address: abdullahlanggeng@gmail.com

I am a national of a non-human primate range-state

Career stage: Research Student (Pre-master)

Provide a 200 word summary of your proposal:

Japanese macaques are the northern-most ranging nonhuman primate and are endemic to Japan. The population at Jigokudani Monkey Park, Nagano, display hot spring bathing behavior (HSBB) for thermoregulation during the cold season. HSBB is known to benefit thermoregulation and reduce stress, but in several human cases it has also been shown to accommodate transmission of heat-resistant waterborne parasites. In turn, parasites can impact host physiological functions, including gut microbiome. Thus, HSBB may expose Japanese macaques to parasites and alter gut bacterial communities both directly and indirectly. At the same time, it may also reduce ectoparasite loads, as several louse species cannot resist high temperatures. To date, there is no study exploring such ecological implications of HSBB related to parasitism and gut microbiome in nonhuman primates. Hence, we aim to investigate the effects of HSBB on gastrointestinal parasite infection risk, gut microbiome, and ectoparasite loads in Japanese macaques. We predict HSBB may increase the risk of intestinal parasite infection if hot spring is a source of infection. Simultaneously, it will alter gut bacterial composition. On the contrary, HSBB is predicted to reduce lice load, and may reduce intestinal parasites if stress reduction buffers immune function.

3 key words: hot spring bathing, parasite, gut microbiome

1. Describe the rationale and significance of your request and how it relates to theory in primatology (1 page maximum)

High latitudes and altitudes can be challenging for wild animals and result in specific behavioral patterns to support survival. Japanese macaques (*Macaca fuscata*) are the northern-most ranging nonhuman primates in the world. They are endemic to Japan and live in diverse habitats, ranging from warm-temperate forests in the south to cold-temperate forests in northern Japan (Fooden & Aimi, 2005). In winter, individuals of a troop residing at the high-altitude site Jigokudani Monkey Park (JMP), Shiga Heights, Nagano, appear to augment thermoregulation through hot spring bathing behavior (HSBB). Recently, this population made headlines when it was found that bathing monkeys experienced decreased stress levels, as measured by faecal glucocorticoids (Takeshita et al., 2018).

Apart from thermoregulation, HSBB and other types of balneotherapy are generally beneficial for health and ameliorating stress among humans. However, there are several cases where hot springs may be detrimental, for instance, in facilitating transmission of heat-resistant, waterborne parasites. For example, hot springs in Taiwan have been contaminated by *Acanthamoeba* spp., which are responsible for keratitis and encephalitis (Hsu et al., 2009). In Thailand, *Naegleria* spp., known to cause meningoencephalitis, and *Acanthamoeba* were distributed in hot springs throughout the country (Lekkla et al., 2005). Such examples highlight clear risks associated with HSBB.

Japanese macaques harbor various parasite species, with intestinal protozoa and helminths, along with ectoparasites, being the most well-known (MacIntosh, 2014). *Balantidium coli*, *Entamoeba* spp., and *Giardia intestinalis* are among the protozoan parasites found in *M. fuscata*. These environmental and waterborne intestinal protozoa may cause diarrheal diseases and be able to resist hot temperatures (Bingham et al., 1979). There remains little data on intestinal protozoa in free-ranging Japanese macaques, and none from JMP, so increasing our knowledge about their diversity and distribution in addition to studying the impacts of HSBB on their dynamics is paramount. Among the myriad influences of parasite infection, gastrointestinal parasites can disrupt host physiological functioning and gut microbiota. The gut microbiome is critical for numerous processes, such as digestion, homeostasis, and immunity. Thus, infection may diminish its functional benefits and exacerbate negative impacts of infection (Moloney et al., 2014).

Assuming the hot spring in JMP serves as a good medium for parasite transmission, while at the same time generally exposing animals to different types of bacteria, HSBB may both directly and indirectly mediate gut microbial dynamics. As it was recently reported that sharing bathtub water among humans contributes to the exchange of gut microbiomes (Odamaki *et al.*, 2019). To our knowledge, such a study on non-human primates has not been conducted. Therefore, we will additionally investigate the impacts of HSBB on gut bacterial composition of Japanese macaques.

Moreover, *M. fuscata* is also susceptible to ectoparasites, such as *Pedicinus obtusus* and *P. eurygaster*. Since louse infestation is also unfavorable, animals have evolved various means of responding to them. For example, primates and other mammals habitually groom (Tanaka & Takefushi, 1993), while birds may sunbath and preen; sunbathing may even concentrate lice for more effective elimination during preening (Blem & Blem, 1993). Interestingly, Harbison & Boughton (2014) found that feather lice preferred host body regions with cooler temperatures, and heat exposures made lice sluggish and often caused mortality. During grooming, Japanese macaques perform conspicuous behavior, whereby they carefully pick and remove an object from the base of the hair and subsequently ingest it, which is directed at louse egg 99% of the time (Tanaka & Takefushi, 1993). They are also reported to have grooming site preferences, corresponding to body parts containing more louse eggs (Zamma, 2002). We currently have no data relating HSBB with ectoparasites, so the current study has the potential to contribute significantly to our understanding of this behavior and its potential outcomes.

2. What are your hypotheses and predictions? (1/2 page maximum)

Our research aims to investigate the effects of HSBB on parasitism and gut microbiota in Japanese macaques inhabiting JMP, Shiga Heights, Nagano, Japan. Several questions guide this research:

- Does HSBB impact gastrointestinal parasite infection risk in Japanese macaques, i.e. do the diversity and intensity of infection vary between bathing and non-bathing individuals?
- Does HSBB impact the gut microbiome of Japanese macaques, i.e. does its alpha, beta, and gamma diversity differ across bathing and non-bathing individuals?
- Does HSBB impact the abundance and distribution of lice on the body of Japanese macaques?

The following hypotheses frame this research to test the above questions:

- Hot spring bathing occurs in winter, so during this season we predict gastrointestinal parasite infection to differ between individuals that bathe (bathers) and those that don't. If hot springs are a source of infection, bathers should be more affected, but if bathing further buffers immunity to infection, the opposite may be found. This may also depend on parasite species. We also predict that bathing will alter gut bacterial composition, either directly through differential exposure to bacterial types or indirectly through mediation of intestinal parasites. Lastly, we predict ectoparasites to be less common or distributed differentially on the body of bathers due to heat exposure; heat should restrict louse activity (e.g. egg-laying) to parts of the body that are rarely immersed (e.g. head, upper back, etc.).
- We predict no differences during summer, when HSBB is absent.

3. What methods, data and statistics will you use to answer your question(s)? Please be specific. (1/2 page maximum)

We have selected a stratified random subset of ca. 16 adult females, half bathers and half non-bathers, determined during the first winter season. Focal continuous sampling is used to record behavior of individuals during 45-minute sessions (ca. 4 sessions/week/subject) to assess (1) HSBB (duration and frequency), (2) nit-picking (active louse egg removal) rates, and (3) grooming sites (during both self- and allo-grooming) preference. Grooming sites are characterized as 9 different body regions following Zamma (2002). To estimate lice abundance, the rate of nit-picking behavior per grooming-minute will be recorded (Duboscq et al., 2016). Agonistic and other affiliative behaviors will be recorded *ad libitum* to assess relative dominance rank and social relationships.

Fecal samples will be collected from focal individuals immediately after defecation and placed into sealable plastic bags. At the earliest opportunity, samples will be partitioned and stored in (1) sodium acetate-acetic acid-formalin (SAF) fixative for endoparasite screening and (2) lysis buffer for gut bacterial analysis and parasite molecular diagnostics. Samples will be stored at room temperature until processing.

Parasites will be identified and quantified using standard protocols and molecular diagnostics. To characterize the gut microbiome, we will follow the methods developed by Hayakawa et al., (2018). After DNA purification, the V3-V4 region of 16S rRNA gene will be amplified. Raw sequences will be processed with a 97% similarity cutoff to classify OTUs. For taxonomic identification, OTUs will be assigned through the ribosomal database project classifier at 50% confidence. All statistical analyses will be performed in the R statistical environment, e.g. using the packages phyloseq and microbiome for OTU diversity estimation and lme4 for modeling the effects of bathing on our response variables in a generalized linear mixed-modeling framework while controlling for variables such as reproductive state, dominance rank, etc.

4. Outline your plans for dissemination of your results (1/3 page maximum)

The outcomes will be submitted to one or more international primatological journals. We will also present at domestic (annual congress of the Primate Society of Japan) and international conferences (e.g. Congress of the International Primatological Society & the International Conference on Animal Parasitology and Entomology).

We will also distribute reports to JMP and other monkey parks across Japan. We believe that the outcomes of our work can inform management decisions related to the potential for disease transmission and risks to tourists, in addition to the animals themselves, so we will be sure to share these results carefully with park staff.

Given the broad appeal of the topic, we also plan to engage with the public about our results, e.g. through Kyoto University's Global Communication Office and the Primate Research Institute's in-house podcast series, The PrimateCast, among other outlets.

5. Provide a timeline for this project (2/3 page maximum)

This project will last for more than one year. The field work will be conducted over two winters and one summer, starting in December 2019. Macaques mostly display HSBB in winter and do not bathe in summer. After each field season, we will transfer the samples from JMP to the Kyoto University Primate Research Institute (KUPRI) in Inuyama, Aichi, for subsequent analyses.

December 2019 – March 2020	**:** First winter field work
April – May 2020	**:** Sample processing and analyses
July – September 2020	**:** Summer field work
October – November 2020	**:** Sample processing and analyses
January – March 2021	**:** Second winter field work
March 2021 – May 2021	**:** Sample processing and data analyses

6. Budget Provide detailed information for all expenditures not to exceed US$1500.00. List other funding sources for this project and amounts.

The budget is for laboratory materials and DNA extraction kits for protozoan and gut microbiome analyses. Field work accommodation and travel expenses are covered via Kyoto University Primate Research Institute's research funding for students and self-funding. We also plan to submit a proposal for a small research grant from the American Society of Primatologists.

Item	Description	Price (USD)
DNA extraction kit	2 kits of QIAamp DNA Stool Mini Kit (50) from QIAGEN	560
Laboratory consumables	reagents, pipettes, tubes, etc.	240
Parasitology staining	trichrome staining kit, acid fast staining	700
	Total	1500

(Optional Section)

Conservation through Community Involvement (CCI)

If you plan to include CCI in your program you may be eligible for an additional award of US$500 to support these initiatives. Please describe your CCI plan below, addressing how these funds will be used and how this will impact conservation in your region. For more information on CCI and suggested CCI practices, please see the Guidelines for Conservation through Community Involvement posted in the publications section of the IPS website. (1/2 page maximum)

7. Literature cited (do not expand this section)

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Zamma K. 2002. *Primates*. 43(1): 41 – 49

8. Brief CV (principal investigator)

ABDULLAH LANGGENG

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EDUCATION

2020 (April) – : M.Sc. student, Kyoto University Primate Research Institute
2019 – current : Graduate Research Student, Kyoto University Primate Research Institute
2011 – 2016 : B.Sc. in Biological Science, Universitas Gadjah Mada, Indonesia

WORK EXPERIENCE

February 2019 : Short-term intern at Kyoto University Primate Research Institute
2016 – 2018 : Research assistant, wildlife trade officer, and field station coordinator assistant at the Little Fireface Project, West Java, Indonesia

SCIENTIFIC PUBLICATIONS AND MEETINGS

Langgeng A., Ardiansyah A., Sigaud M., Birot H., Imron M.A., Nijman V., & Nekaris K.A.I. 2019. An inventory of non-native birds at Indonesia's bird markets. Symposium on Integrative Biology II. Kyoto, Japan.

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Nijman V., **Langgeng A**., Ardiansyah A., Birot H., Imron M.A., & Nekaris K.A.I. 2018. Grosbeak starling *Scissirostrum dubium* in the cage-bird trade in Indonesia. *Birding Asia*. 30: 8 – 9

Nijman V., **Langgeng A.**, Birot H., Imron M.A., & Nekaris K.A.I. 2018. Wildlife trade, captive breeding and the imminent extinction of a songbird. *Global Ecology and Conservation*. E00425

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Farukh RAP., Yusqi T., Gian AP., Muhammad Z., **Langgeng A**., Farah MF., Sulhan E., Ika NC., Khoirun AN., Gravinda W., Anita DK., Dita KM., Denya S., Risnelli. 2014. *Keanekaragaman Moluska di Pantai Krakal, Gunung Kidul, Yogyakarta*. Seminar Nasional Masyakarat Biodiversitas Indonesia. Depok, Indonesia.

Muhammad Z., Cahyo A., **Langgeng A**., Farah MF.,& Sulhan E. 2013. *Biodiversity of Mollusks at Ela-Ela Beach, Sekotong Lombok Barat Indonesia*. The 3rd International Conference on Biological Science. Yogyakarta, Indonesia.

FUNDINGS

2019 – present : Monbukagakusho (MEXT) Scholarship
2017 : WWF Russel E. Train Education for Nature Professional Development Grant
2015 : JASSO Scholarship

Send this application AS A SINGLE PDF DOCUMENT, named "LAST NAME, First name.pdf" to: Prof Marina Cords (marina.cords@columbia.edu)