Research Activity Report
Supported by "Leading Graduate Program in Primatology and Wildlife Science"
(This report must be submitted within two weeks of the end of your PWS-sponsored trip.)

	2025. 11, 12
Affiliation/Position	Wildlife Research Centre/M1
Name	Minsheng Huang

1. Country/location of visit

Japan/Kyoto University South Campus, Wildlife Research Center

2. Research project

Advanced laboratory skills in field biology

3. Date (departing from/returning to Japan)

2025.11.04 – 2025.11.07 (4 days)

4. Main host researcher and affiliation

Prof. Murayama and Dr. Sato / Wildlife Research Centre of Kyoto University

5. Progress and results of your research/activity

During 2025/11/04 to 2025/11/07, we had a course of advanced laboratory skills in field biology in Kyoto University south campus and laboratory in WRC, instructed by Prof. Murayama, Dr. Sato and assistance from Xorlali, Fadel, Nakamura.

Day 1:
We went to collect the feathers in Kyoto University campus. We totally collected fourteen bird feather samples from different campus. Although we also went to Yoshida Shrine, no samples were found there. Ozasa had collected feathers from the Palace before the course began. After that, we also got two samples from Ishigaki so we had sixteen samples together into the next step.

Figure 1. Sample collection in campus

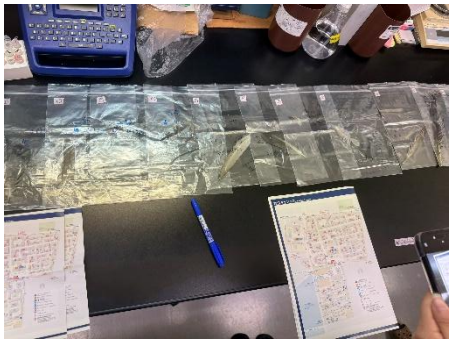

Figure 2. Sixteen feather samples

On afternoon, we started the lab work of species identification with mtDNA COI in the gene laboratory inside WRC. We extracted DNA from feather samples, measured DNA concentration and then put them into the PCR step. PCR conditions were set as PCR reaction (LA55C40): 95℃ 2min (95℃ 30s, 55℃ 30s, 74℃ 1min) ×40, 74℃ 10min, 10℃ until Day 2.

Day 2:
On the second day, we retrieved our samples from PCR machine and checked its success by Gel electrophoresis under the instruction of Xorlali. We mixed samples with blue dye and pipetted them carefully into the entrance of gel and then turn on the electricity (100V for 20 minutes). DNA samples were negatively charged so they will move from negative electrode to positive electrode. Longer DNA molecule would move slower so that different bands would appear on gel after the electrophoresis. After the electrophoresis, we observe the gel under the UV light.

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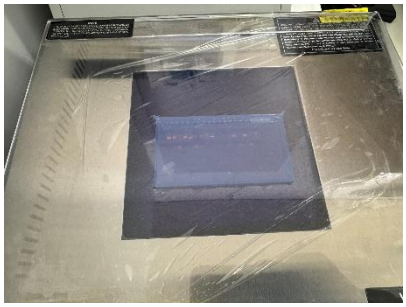

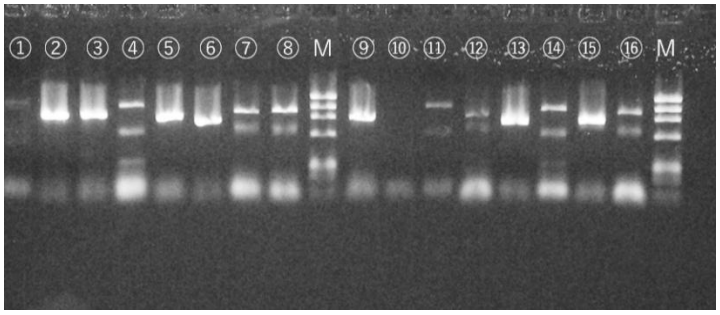

Figure 3&4. Results of gel electrophoresis

As a result, some samples showed clear bands on the gel while some not. To some samples, at least two bands were found on the gel, which means that sample might be contaminated when we collected them or during the analysis or non-specific amplification might have happened because DNA volume/quality from feathers was not enough.
We continued our analysis and entered the next step to do purification of PCR products. After the purification, we add sequencing reaction buffer into the PCR products and started sequencing reaction (SEQ): 96℃ 1min (96℃ 10s, 50℃ 5s, 60℃ 1min) ×25, 10℃ until we picked it up again on afternoon.
After lunch, we did the ethanol participation and sequencing as the last step.
During this, Prof. Murayama and Dr. Sato also taught us how to do sex identification to birds. Just like human, birds also have their own sex chromosome. Unlike XX for female and XY for male to human. Birds have ZZ for male and ZW for female. They have different length in PCR porduct from Z and W chromosomes respectively. So, PCR products would move with different speed in gel electrophoresis. Female birds would show two bands on the gel while male birds only show one band. (We did not try to do it by ourselves, just learn that as a lecture.)
Finally, we checked the sequence of the samples on Finch TV and then did the species identification with some website online.

Location	**Estimated Species**	**Confirmed Species**	PCR result
Yoshida South	Dove	Rock dove	×
Yoshida South	Dove	Oriental turtle dove	✓
Yoshida South	Dove		✓
Yoshida South	Dove	Sooty tern	‖
Yoshida South	Dove	Rock dove	✓
Yoshida South	Dove	Rock dove	✓
Yoshida South	unclear_small	Carrion crow	‖
Yoshida South	Dove?_white		‖
Yoshida South	Dove	Rock dove	✓
Yoshida South	Crow		×
Yoshida South	Crow	Carrion crow	‖
Palace	Crow	Rock dove	‖
Palace	Kite	Rock dove	✓
Yoshida South	unclear_small	Sooty tern	‖
Ishigaki	Crow		✓
Ishigaki	Dove	Sooty tern	‖

Table 1. Results of sequencing analysis

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Twelve samples of sixteen were successfully identified even though some of them were failed to show any bands during the gel electrophoresis. As the results showed, we collected samples from rock doves, carrion crows, oriental turtle doves and surprisingly sooty terns. According to teachers and Nakamura, this species is not so familiar around the Kyoto University campus. But we indeed collected their feather samples in south campus. It was considered that it might be brought here from other places by wind or typhoon but true reasons remain unknown.

There are not many opportunities for me to do some work inside laboratory since I usually do some field work aboard and analyze data just by applications with computer. It was fun for to attend this course held by Prof. Murayama and Dr. Sato. I also learnt useful skills and some principle of the DNA analysis. I am confident that it would help me a lot to understand more during the lecture and others' presentation about DNA lab work from now on.

6. Others

I would like to thank Prof. Murayama and Dr. Sato to hold this course for us. I also would like to thank all the members who cooperated with me to complete this course and Fadel, Xorlali and Nakamura's instruction during our work. And thank PWS program to give us a chance to attend this course.