

Research Activity Report
Supported by “Leading Graduate Program in Primatology and Wildlife Science”
 (Please be sure to submit this report after the trip that supported by PWS.)

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Affiliation/Position	Wildlife Research Center/D1
Name	Louzamira Biváqua

1. Country/location of visit
Kyoto, Japan.
2. Research project
Endangered and endemic Yakushima fern species (<i>Haplopteris yakushimensis</i>) gametophyte found in the Hanaage basin?
3. Date (departing from/returning to Japan)
2014. 05. 29 – 2014. 06. 06
4. Main host researcher and affiliation
Dr. Hirotooshi Sato, Kyoto University.
5. Progress and results of your research/activity (You can attach extra pages if needed)
Please insert one or more pictures (to be publicly released). Below each picture, please provide a brief description.
<p>During my visit at Kyoto University, I conducted research on genetics of ferns. The objective of the project was to identify the species composition of ferns in Hanaage River (one of the collection sites in Yakushima) using DNA barcoding technique.</p> <p>For our study we used 48 samples of gametophytes (n). First we extracted DNA from each sample for PCR, which amplified partial regions of chloroplast rbcLgenes (total length: 670 bps). After this step, was performed the electrophoresis to confirm the PCR results and then Cycle sequencing was done (Figure 01). For the analyzes we used the software MEGA6. We tested 3 different primers, but only one - primer Sequence: rbcLaF (5'-ATGTCACCACAAACAGAGACTAAAGC-3 ') - proved appropriate.</p> <p>As a result, we identified 7 species and nine genera of ferns through genetic analysis. Four gametophyte's samples were identified as <i>Haplopteris forrestiana</i>, which could be <i>Haplopteris yakushimensi</i>, the recently reported and endangered species endemic to Yakushima.</p> <p>After the course we presented our results as posters at 3rd International Seminar on Biodiversity and Evolution in Kyoto (Figure 02).</p> <p>In a general way, this genetics course was very interesting to me. I was satisfied with the opportunity to develop a field work at the beautiful Yakushima, and beyond the still be able to, in a short time frame, develop the genetic research and reach satisfactory results.</p>

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6. Others



Figure 01. Conducting eletrophoresis at the genetics laboratory.

Endangered and endemic Yakushima fern species (*Haplopteris yakushimensis*)' gametophyte found in the Hanaage basin?

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Introduction

Fern = seedless vascular plant

- Has vascular bundles for conveying water & nutrition and supporting body
- Reproduces via spores, which are sometimes dispersed over long distances by wind
- Distributions of sporophytes and gametophytes may differ
- Gametophytes, compared to sporophytes, are not well understood; intense studies have only recently begun
- Gametophytes are under 10mm, with three different types of shape: cordiform (heart shape), ribbon-like and filamentous
- It is impossible to distinguish between species by the morphology of gametophytes
- but DNA sequencing can tell!

Yakushima's richness of ferns

- Half of the >600 species distributed in Japan are found in Yakushima
- Hanaage basin is especially well-known for its wet environment and species richness
- Haplopteris yakushimensis* is a recently reported new species endemic to Yakushima, said to be found only in one location** (National Museum of Nature and Science, press release, Feb. 6th, 2014), which was formerly regarded as *Haplopteris forestiana*
- Haplopteris yakushimensis* is endangered** due to deforestation (Ministry of the Environment, URL on bottom) or herbivory by overabundant deer (National Museum of Nature and Science, same as above)

Objective

To reveal and compare the species compositions of fern gametophytes & sporophytes in the Hanaage basin

Materials & Methods

(Yakushima field training course)

Sampling site: Hanaage basin (E.88°18'00"E, lat. 130°23'00"N), Yakushima Island, Kagoshima

Sampling day: May 22nd, 2014

Sporophytes (2n)
Sample collection → herbarium → identification (by morphology)

Gametophytes (n)
Sample collection → preservation in silica gel (for DNA analysis) & ethanol (for evidence)

(Genome training course)

Gametophyte samples for DNA analysis: 48

Process:
DNA extraction → PCR → Electrophoresis → Purification of PCR products
 → Cycle sequencing → Genetic analyzer (ABI3130)
 → Analysis (MEGA5)

PCR: amplified partial regions of chloroplast *rbcl* genes (total length: 670 bps) (Ishizuka et al., 1994)

PCR Primer: *rbcl*-af & *rbcl*-ar

Sequence primer: *rbcl*-af
 (5'-ATGTCACCACAACAGAGACTAAAGC-3')

Results

Fig. 1. Neighbor-joining tree based on *rbcL* genes of gametophytes collected in the Hanaage basin.

- Obtained 38 DNA sequences
- Found 12 operational taxonomic units (OTU)
- Identified 7 species & 9 genera
- All genera found as gametophytes were also found as sporophytes, most of which are widely distributed throughout Yakushima, although...
- 4 gametophyte samples were identified as *Haplopteris forestiana*, which could be *Haplopteris yakushimensis* (Fig. 1, circled in red), the recently reported new & endangered species endemic to Yakushima
- Sporophytes of the above were not found
- Above samples had 12 different base pairs from the common *Haplopteris flexuosa* (out of 566 bps) (handout available)
- 1 sample, *Haplopteris* sp. (Fig. 1, circled in blue), had 3 different base pairs from the possible *Haplopteris yakushimensis*
- another new species?

Discussion & Conclusion

- All genera of gametophytes found in Hanaage basin were also found as sporophytes, most of which are commonly found throughout Yakushima
- We found 4 gametophytes that could be *Haplopteris yakushimensis*, the recently reported endangered species endemic to Yakushima (out of 38 DNA sequences obtained)
- We found one sample, *Haplopteris* sp., that could be a new species closely related to *Haplopteris yakushimensis*
- We collected samples for only one day, analyzed only 48 samples and obtained only 38 DNA sequences
- With considerably little effort, we found 4 gametophytes of the supposedly difficult-to-find endangered species, *Haplopteris yakushimensis*, said to be found only in one location (at least as sporophytes)
- If we search wider, we may find more new or rare/endangered species!

Figure 02: Poster presented at the International Seminar.