

**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.)

2016.06.17

<b>Affiliation/Position</b>	Centre for Ecological Science, Indian Institute of Science, Bangalore, India/JRF
<b>Name</b>	Kunal Deepak Arekar

<b>1. Country/location of visit</b>
Inuyama, Japan
<b>2. Research project</b>
To determine the phylogenetic relationships between different species of figs ( <i>Ficus</i> ) and fig wasps in
<b>3. Date (departing from/returning to Japan)</b>
2016.05.30 – 2016.06.03 (05 days)
<b>4. Main host researcher and affiliation</b>
1. Professor Takashi Hayakawa (Primate Research Institute, Kyoto University) 2. Professor Munehiro Okamoto (Primate Research Institute, Kyoto University)
<b>5. Progress and results of your research/activity</b> (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.
I participated in the genome course that was conducted at the Primate Research Institute at Inuyama, by Dr. Hayakawa. The aim of this course was to determine the phylogenetic relationship between the fig species and fig wasp species at Yakushima island and to see if there are any signs of coevolution among the figs and fig wasps.
The samples were collected during our field session at Yakushima island; there were 8 plant samples and 48 insect samples. Over the next few days we extracted, PCR amplified and sequenced DNA from these samples. We also analyzed the sequences and build phylogenetics trees. The DNA extraction was carried out using commercially available kits like Qiagen. We used microbeads to crush the samples into fine powder. Then we PCR amplified this extracts using specific primers. We sequenced atpB-rbcL chloroplast gene for the fig samples and 28S rRNA nuclear gene for insects, both these markers are non-coding. After this we purified these PCR products and sequenced the DNA using Sanger sequencing technique. The sequences were edited and aligned using commercially available softwares like FinchTV. These sequences were aligned using Muscle algorithm in MEGA 5. Phylogenetic tree reconstruction was performed in MEGA 5.2 by Neighbour Joining (NJ) method.
Our results showed no coevolution between the figs and fig wasps. This could be an artifact of less number of samples or another possibility is short length of the DNA marker or both. However, it could also mean that the fig and fig wasps in Yakushima island show no coevolution. But, to determine this we need more number of samples and more number of markers. We were able collect only a few samples during the time we had. If more time is invested, it is possible to collect more number of samples to address this issue. The preliminary trees showed some interesting results. Dioecious plants like <i>F. pumila</i> and <i>F. erecta</i> have a specific fig wasp species as pollinator like <i>Wiebesia pumilae</i> and <i>Blastophaga nipponica</i> respectively. On the other hand, there are multiple species of wasps that occupy the monoecious species of <i>Ficus</i> like <i>F. microcarpa</i> and <i>F. superba</i> (Figure 1). From the phylogenetic tree (Figure 2), we can see distinct clades for <i>F. microcarpa</i> , <i>F. superba</i> and <i>F. erecta</i> . But, the relationship between <i>F. pumila</i> and <i>F. sarmentosa</i> is not clear. There might be a possibility that this can be a species complex. By adding more number of samples and maybe by increasing the number of markers, we might be able to resolve this complex.
Although I have been working in the field of phylogenetics for past four years, I learned many new things in this course which will benefit me for my own work. Use of zirconia beads for DNA extraction was something new for me; I have never used it before. Also, back in India, we outsource the sequencing part to other institute or company. During this course, I learn how to prepare samples for sequencing and how to load them inside a sequencer. Another new technique that I learned is to use harmless compounds like GelGreen to stain the gels, as a substitute for EtBr (Ethidium Bromide; a carcinogenic gel staining solution)

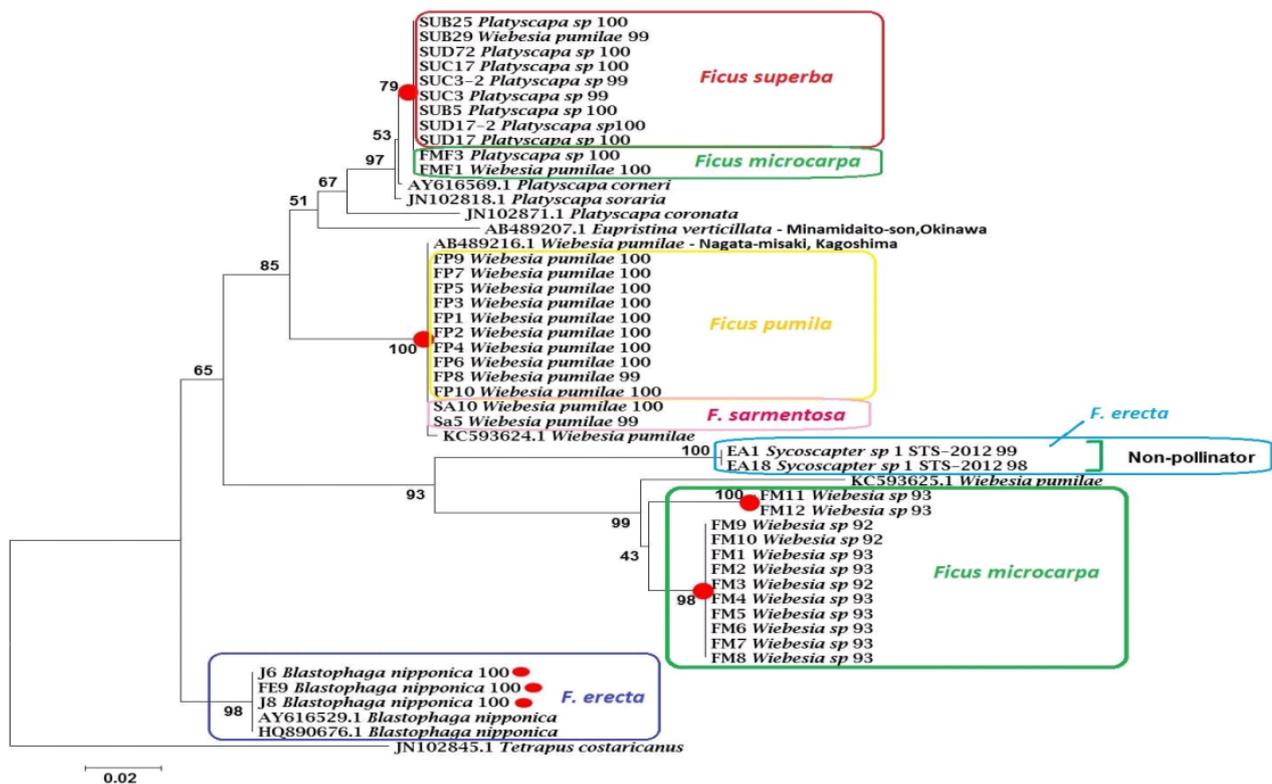
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during gel electrophoresis.

It was an amazing learning experience for me. I am very grateful for the guidance provided by all the lecturers and tutors. I am also thankful to all the participants who helped me during this whole course.

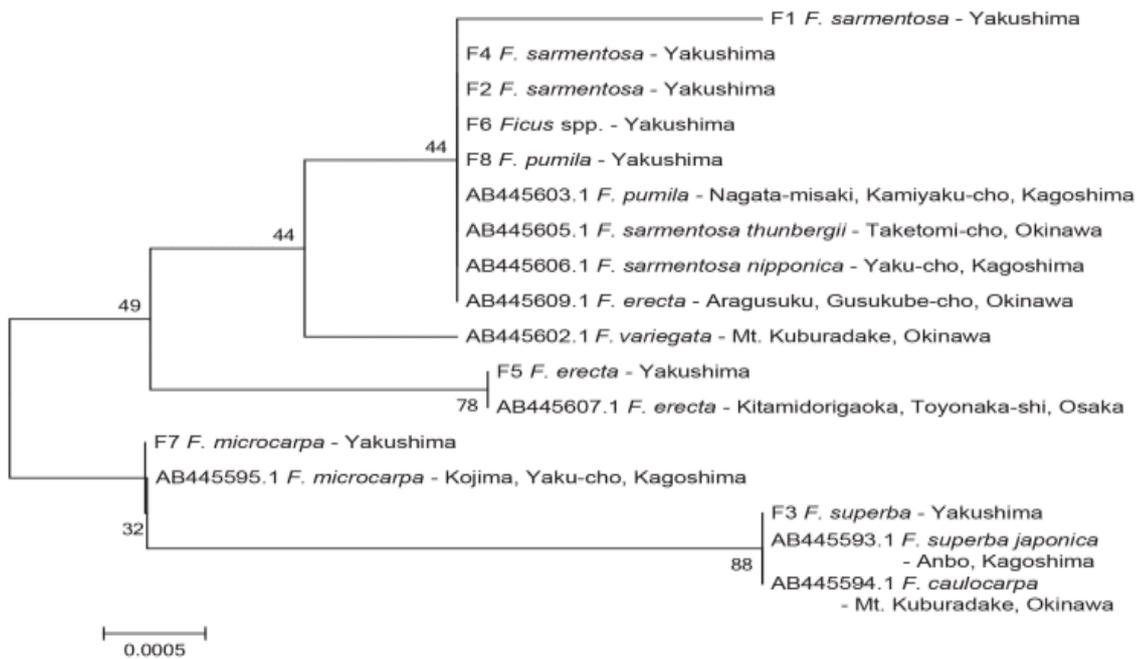
**6. Others**

While in Inuyama, we also visited the Japan Monkey Centre (JMC). They have a very good collection of monkeys from all over the world. It was very exciting to see some of these rare and endangered monkeys right in front of you. We also visited the chimpanzee facility at the PRI where they study cognition and behavior of these apes. It was very impressive to see such a large collection of primates in one place.



**Figure 1: Neighbour-Joining tree of Fig wasps using 28S rRNA nuclear region**

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**Figure 2: Neighbour-Joining tree of *Ficus* species using *atpB-rbcL* chloroplast gene**