DNA metabarcoding of springtails (Collembola)

Seikoh Saitoh1, Hiroaki Aoyama3, Saori Fujiiz, Haruki Sunagawa3, Hideki Nagahama1, Masako Akutsu4, Naoya Shinzato1, Nobuhiro Kaneko2, Taizo Nakamori2

1 Tropical Biosphere Research Center, University of the Ryukyus, Japan; 2 Department of Environment and Natural Sciences, Graduate School of Environment and Information Sciences, Yokohama National University, Japan; 3 Okinawa Prefectural Agricultural Research, Japan; 4 Department of Electrical Engineering and Computer Science, School of Industrial and Welfare Engineering, Tokai University, Japan

Abstract:
- We developed novel, quantitatively superior protocols for DNA metabarcoding of Collembola, a soil microarthropod group.
- Degenerated primers for mitochondrial cytochrome c oxidase subunit I (mtCOI) and 16S ribosomal RNA (mt16S) genes were designed and screened on the basis of their ability to amplify the genes, irrespective of the collembolan species.
- DNA libraries of collembolan community samples were prepared from amplicons of the genes by ligation with adaptors for 454 technology.
- After normalization, the sequence abundances for each collembolan species showed linearity to a number of individuals included in the community samples. Both the mt16S and mtCOI data showed good linearity (R = 0.91–0.99).

Introduction:
- Springtails (Collembola) are a major group of soil microarthropods that mediate food webs during the decomposition process (Fisher 2002, Pedobiologia).
- Collembola are conventionally identified to the species level by microscopic examination of their morphological features; therefore, the community assessment of this group has been time-consuming till now.
- The application of next-generation sequencing technology to the community assessment (DNA metabarcoding; Taberlet et al. 2012 Mol. Ecol) of this group could be an appropriate solution.
- Although several DNA metabarcoding methods for microscopic animals have been published till date, their use has been limited because they tend to produce poor quantitative results, which is mainly due to bias during PCR amplification (e.g., Ramirez-Gonzalez et al. 2013 PLoS ONE).
- More precise quantitative identification methods are thus required.

Materials and Methods: Primer design and screening

- Finding the conserved regions in mtCOI and mt16S genes based on published collembolan mitogenomes.
- Designing as many degenerated primers as possible (indicated by arrows).
- Selecting the best primer pairs for each gene based on the examination of PCR amplification using collembolan species from various families.

Preparation of DNA libraries for 454 technology

- Community samples were homogenized and the total DNA was extracted using the Qiaquick DNA Blood and Tissue Kit.
- PCR was performed with the selected primer pairs with multiplex identifier (MID) tags using KAPA HiFi HotStart ReadyMix (50°C for 2 min, 20-25 cycles at 94°C for 1 min, 55°C for 30 s, and 72°C for 30 s, followed by the final extension step at 72°C for 1 min).

Data processing

- The amplified gene regions were sequenced in both directions.
- The output sequences were grouped into operational taxonomy units (OTUs) with a 90% similarity threshold.
- Taxonomy was determined using a reference sequence database constructed by Sanger sequencing.

Results and discussion: I. Assessment using simulated community samples

(a) Animals
(b) mtCOI
(c) mt16S

Table 1 Collembolan community in a forest in Kamigamo (Kyoto, Japan) examined by morphology and sequencing (n = 10)

Conclusion: Both methods developed in the present study showed good linearity to the number of individuals and were effective in assessing the biodiversity of Collembola.