



DNA metabarcoding of springtails (Collembola)

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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 ($R = 0.91\text{--}0.99$).

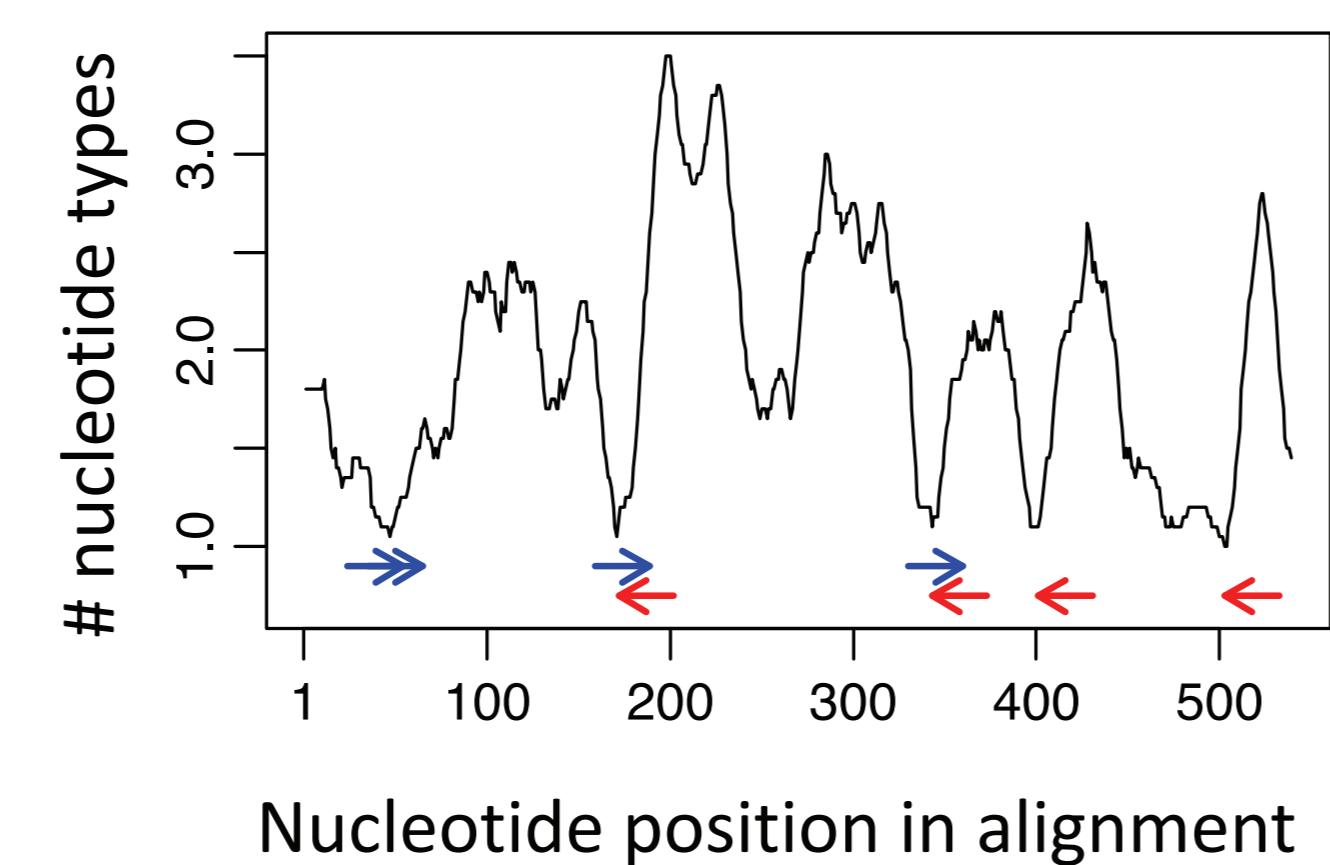
Introduction:



- Springtails (Collembola) are a major group of soil microarthropods that mediate food webs during the decomposition process (Filser 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.

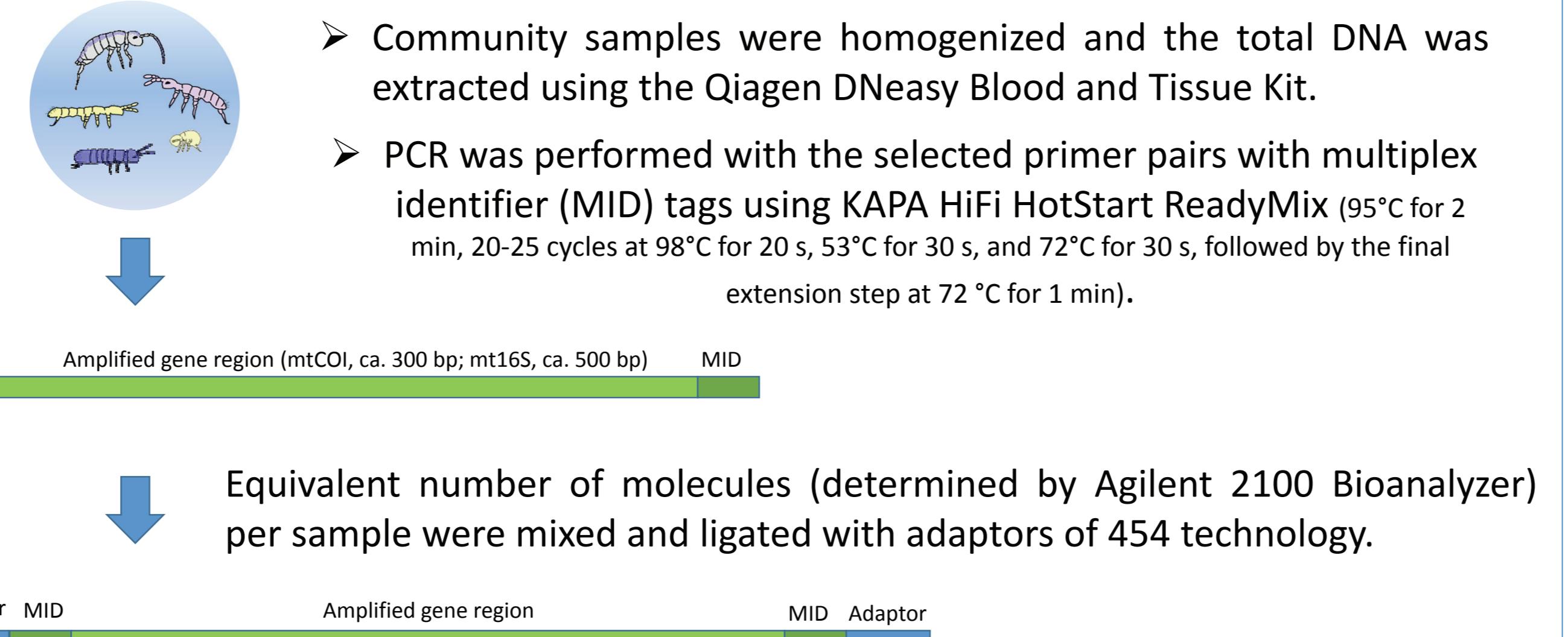
Materials and Methods:

Primer design and screening

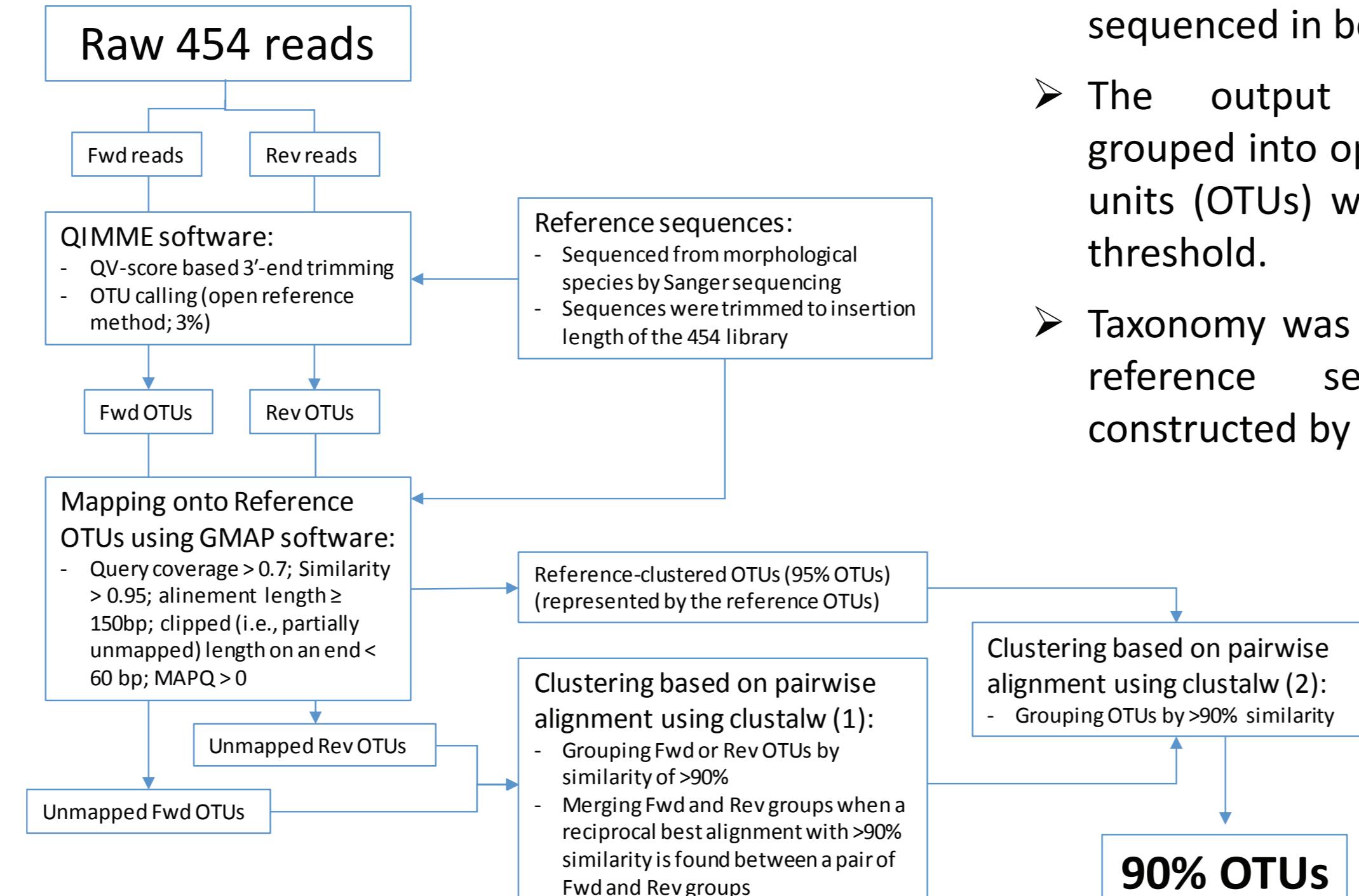


- Finding the conserved regions in mtCOI and mt16S genes based on published collembolan mitogenomes.
 - Designing as many degenerated primers as possible (indicated by arrows).
 - Selecting the best primer pairs for each gene based on the examination of PCR amplification using collembolan species from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

Preparation of DNA libraries for 454 technology



Data processing



Acknowledgements:

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Results and discussion

I. Assessment using simulated community samples

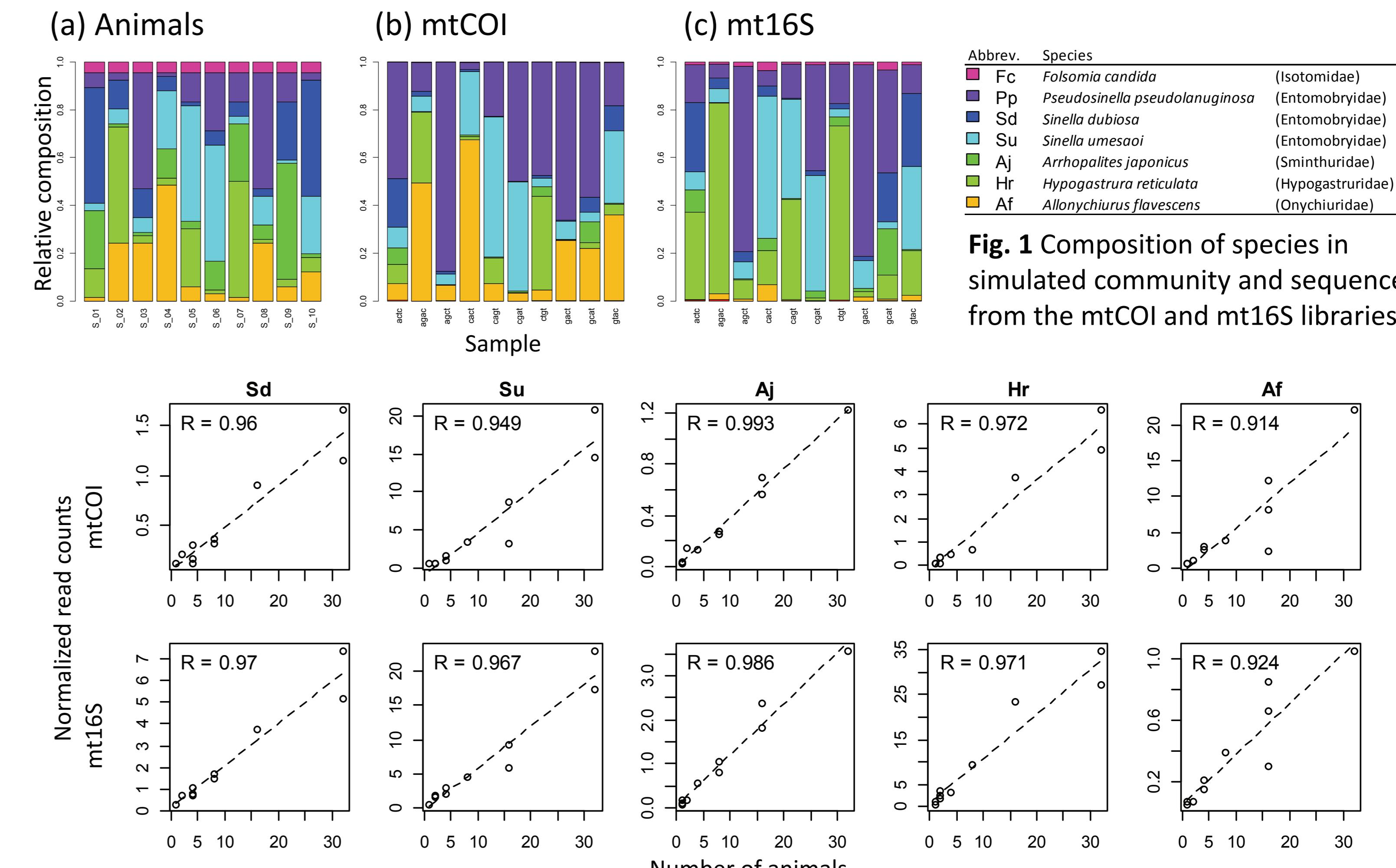


Fig. 2 Relationship between number of animals in simulated community samples and normalized read counts per species. *Pseudosinella pseudolanuginosa* was used as an internal standard.

II. Assessment using natural community samples

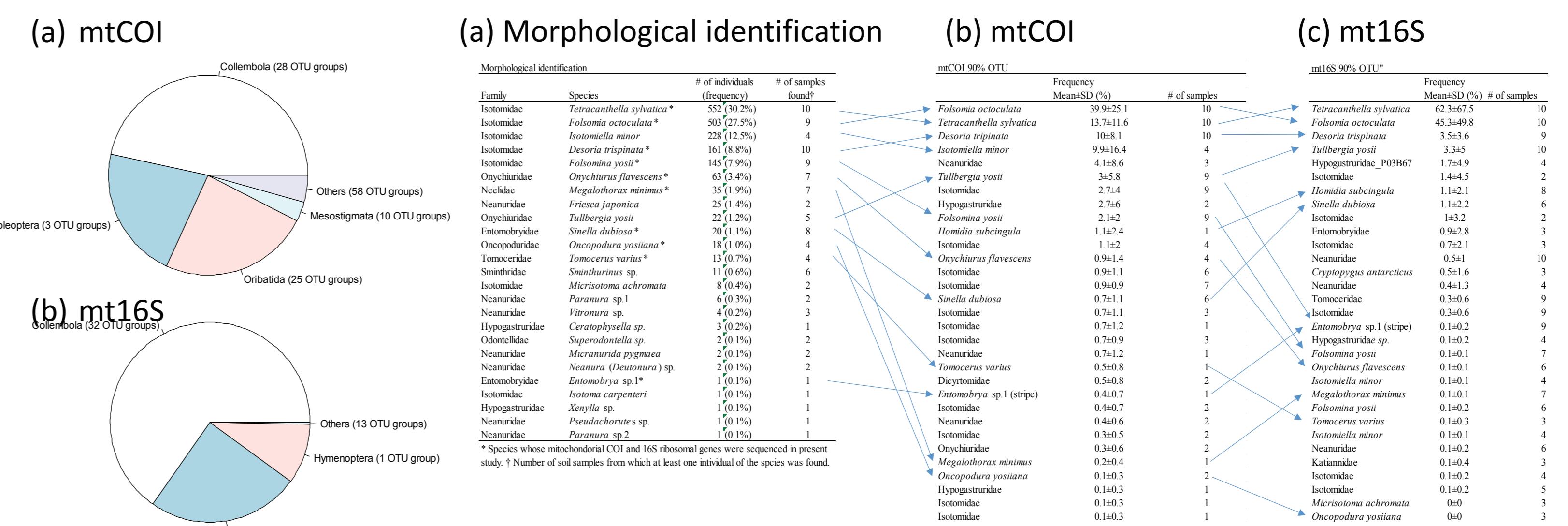


Table 1 Collembolan community in a forest in Kamigamo (Kyoto, Japan) examined by morphology and sequencing ($n = 10$)

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.