ノート (Note)

Isolation of Pristionchus bucculentus from the large mushroom beetle, Encaustes praenobilis

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An entomophilic (insect phoretic) nematode, Pristionchus bucculentus Kanzaki, Ragsdale, Herrmann, Röseler & Sommer (Rhabditida: Diplogastridae) was originally described from the dissected body of a shining mushroom beetle, Episcapha gorhami Lewis (Coleoptera: Erotylidae), found on a Basidiomycota fungus occurring on dead wood in Sapporo, Hokkaido, Japan (Kanzaki et al. 2013). The species was reisolated from the large mushroom beetle, Encaustes praenobilis Lewis (Coleoptera: Erotylidae), during a field survey of insect-associated nematodes, and the isolation information is described as a new locality and a new carrier. Molecular barcode information (near-full-length of 18S ribosomal RNA, D2/D3 expansion segments of 28S ribosomal RNA and the partial sequence of mitochondrial cytochrome oxidase subunit I) is presented.

One male and three female adults of E. praenobilis were hand-sampled on August 9, 2013 in the Shirakami mountains, Nishimeya, Aomori, Japan. The insects were brought back to the laboratory, and dissected alive under a stereomicroscope to examine the associated nematodes. After the dissection, the dissected bodies were placed individually on 2.0% water agar (WA) plates, and kept at room temperature. Subsequently, the plates were examined once a week for 1 month. Although the nematodes were not recognized during the dissections, they propagated on all four insect bodies, feeding on bacteria 1 week after the dissections. The mouth and pharynx morphology of propagated nematodes were observed under light microscope to determine their feeding habitats. The nematodes were confirmed as bacteria-feeders, thus, they were transferred to NGM agar to establish laboratory cultures. The cultures were observed under light microscopy for morphological identification, using the method of Kanzaki (2013). DNA samples were prepared using the method of Tanaka et al. (2012), and the molecular sequences were determined following the methods in Kanzaki and Futai (2002) and Ye et al. (2007). The species diagnostic characters, i.e., thin and membrane-like per- and interradial cheilostomatal plates of males and females (Fig. 1), and arrangement of male genital papillae and phasmids, i.e., v1, v2d, v3, v4, ad, phasmid, v5, v6, v7, pd, from anterior (Fig. 2) were identical to the original description of *P. bucculentus* (Kanzaki et al. 2013). The newly determined molecular barcodes were deposited in GenBank with the accession numbers AB852581 (ca. 1.6 kb of near-full-length SSU), AB852582 (ca 750 bp of D2/D3 LSU), and AB852583 (ca. 700 bp of mtCOI), and the corresponding part of near-full-length SSU was identical to previously determined ca. 500 bp of speciesspecific molecular barcode (KC463832) (Kanzaki et al. 2013).

Pristionchus bucculentus was originally described as an associate of E. gorhami (Kanzaki et al. 2013). E. gorhami and the new carrier (host) E. praenobilis are close taxonomically, and known as "mushroom beetles"; although they belong to different genera, they have similar preferred habitats, i.e., they feed on

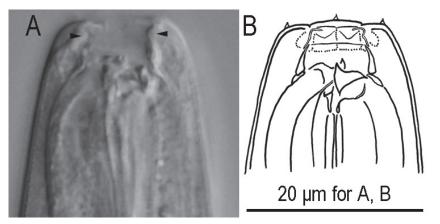


Fig. 1. Right lateral view of stomatal part of Pristionchus bucculentus. A: Micrograph; B: Schematic drawing. Cheilostomatal plates are suggested by arrows.

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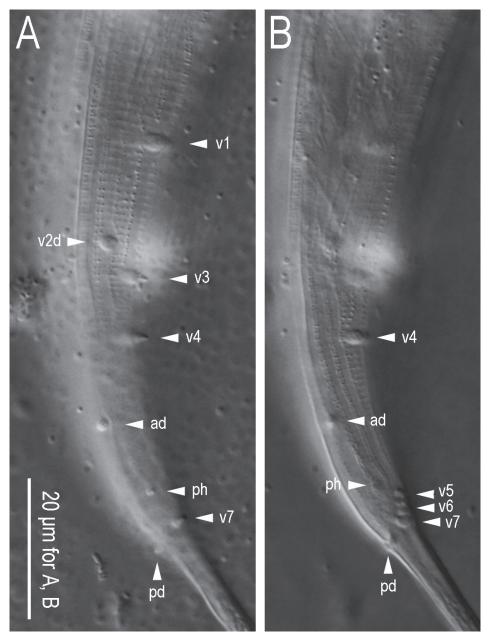


Fig. 2. Male tail characters of *Pristionchus bucculentus*.A, B: Right lateral view of male tail in different focal planes.Genital papillae (v+number, ad, pd) and phasmid (ph) are suggested by arrows.

Basidiomycota fungi occurring on dead wood (Kurosawa et al. 1985). The nematode species might be associated with a wide range of mushroom beetles, and prefer habitat conditions that are suitable for mushroom beetles.

References

- Kanzaki, N. (2013) Simple methods for morphological observation of nematodes. Nematol. Res. 43, 15-17.
- Kanzaki, N. and Futai, K. (2002) A PCR primer set for determination of phylogenetic relationships of *Bursaphelenchus* species within *xylophilus* group. Nematology 4, 35-41.
- Kanzaki, N., Ragsdale, E.J., Herrmann, M., Röseler, W. and

Sommer, R.J. (2013) *Pristionchus bucculentus* n. sp. (Rhabditida: Diplogastridae) isolated from a shining mushroom beetle (Coleoptera: Scaphidiidae) in Hokkaido, Japan. J. Nematol. 45, 78-86.

- Kurosawa, Y., Hisamatsu, S. and Sasaji, H. (eds.) (1985) The Coleoptera in Japan in color. Hoikusha, 500 pp.
- Tanaka, R., Kikuchi, T., Aikawa, T. and Kanzaki, N. (2012) Simple and quick methods for nematode DNA preparation. Appl. Entomol. Zool. 47, 291-294.
- Ye, W., Giblin-Davis, R. M., Braasch, H., Morris, K. and Thomas, W.K. (2007) Phylogenetic relationships among *Bursaphelenchus* species (Nematoda: Parasitaphelenchidae) inferred from nuclear ribosomal and mitochondrial DNA sequence data. Mol. Phylogenet. Evolut. 43, 1185–1197.