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SHORT NOTE

Genetic diversity of Japanese Dugesiidae (Platyhelminthes, Tricladida, Paludicola) studied by comparisons of partial 18S rDNA

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The specimens used in this study were collected from seven localities in Japan (Table1). The total DNA prepared from 14 living specimens was extracted according to procedures described in DE Vos & DICK (1). To isolate partial 18S rDNA, Polymerase Chain Reaction (PCR) was used with two primers (5'-TACTGTTGATCCTGCCAGTA-3' AND 5'-ATTACCGCGGCTGCTGGCACC-3', (2)). Amplified DNA fragments were purified with Wizard PCR Preps DNA Purification System (Promega), and were cloned in the pGEM-T Vector (Promega). Positive clones were confirmed with a colony PCR method. The purified PCR fragments were sequenced by the dideoxynucleotide termination method with a HITACHI SQ-5500 sequencer using the Texas Red labelled M13 primers.

TABLE 1

Japanese Dugesiidae sampled for this study

Species	Number of specimens	Number of clones	Locality
D. japonica	2	8	Hirosaki, Aomori Pref.
	1	4	Hashikami, Aomori Pref.
	1	2	Asahikawa, Hokkaido
	2	2	Iruma River, Gifu Pref. (1)
Dugesia sp.	4	9	Narutani River, Mie Pref.
D. ryukyuensis	2	2	Chinen Village, Okinawa Pref. (2)
D. tigrina	2	4	Nagoya, Aichi Pref. (3)

(1) GI strain established by Himeji Institute of Technology.
(2) OH strain established by Hirosaki University.
(3) from laboratory culture in Nagoya University. Details in KAWAKATSU et al. (1985).

The partial 18S rDNA sequence data are shown in Fig. 1. The sequence of *Dugesia japonica* was consistent with all clones from three localities (Hirosaki, Hashikami and Asahikawa). In the specimens collected from Gifu, the sequences of two clones, from two specimens, were different, but another was identical to those found in the other localities. Two types of partial 18S rDNA (*D. tigrina* 1 and *D. tigrina* 2) were also isolated from *D. tigrina* collected in Nagoya (3). The phenomenon has been reported from other populations of *D. tigrina* (4). Among the clones of *Dugesia* sp. from Mie we recognized five distinct sequences (*Dugesia* sp.M1-M5); clones M1 and M2 share 99.4% of sequence identity, the highest among the sequences that were compared among the Dugesiidae characterized here.

To investigate the phylogeny of Japanese Dugesiidae we reconstructed a phylogenetic tree using neighbour joining method as implemented in PHYLIP program version 3.5c (5). Fig. 2 illustrates the phylogenetic tree and the bootstrap values at all nodes. We suggested that M1 and M2 clones belong to type II 18S rDNA, M5 belongs to type I because it corresponds to the type I sequence of *D. japonica* from Gifu, and M3 and M4 perhaps belong to Type II. Of those specimens collected from the Mie population, the frequency of clone types is as follows, (clone type: number): (M1:3), (M2:2), (M1+M5:1), (M3+M4: 1). In *Dugesia ryukyuensis*, only type II of 18S rDNA sequence could be isolated.

KATAYAMA et al. and CARRANZA et al. have already reported the 18S rDNA sequences of *Dugesia japonica* (6, 7). We compared these sequences with our data. The alignments show that our sequences were different from the sequences and we believe this is due possibly to differences in sequencing method and/or genetic variation. The direct sequencing method is not appropriate for Dugesiidae because many species of *Dugesia* have two types of 18S rDNA (4, 8). We detected genetic diversity among *Dugesia* sp. from Mie and *D. japonica* from the

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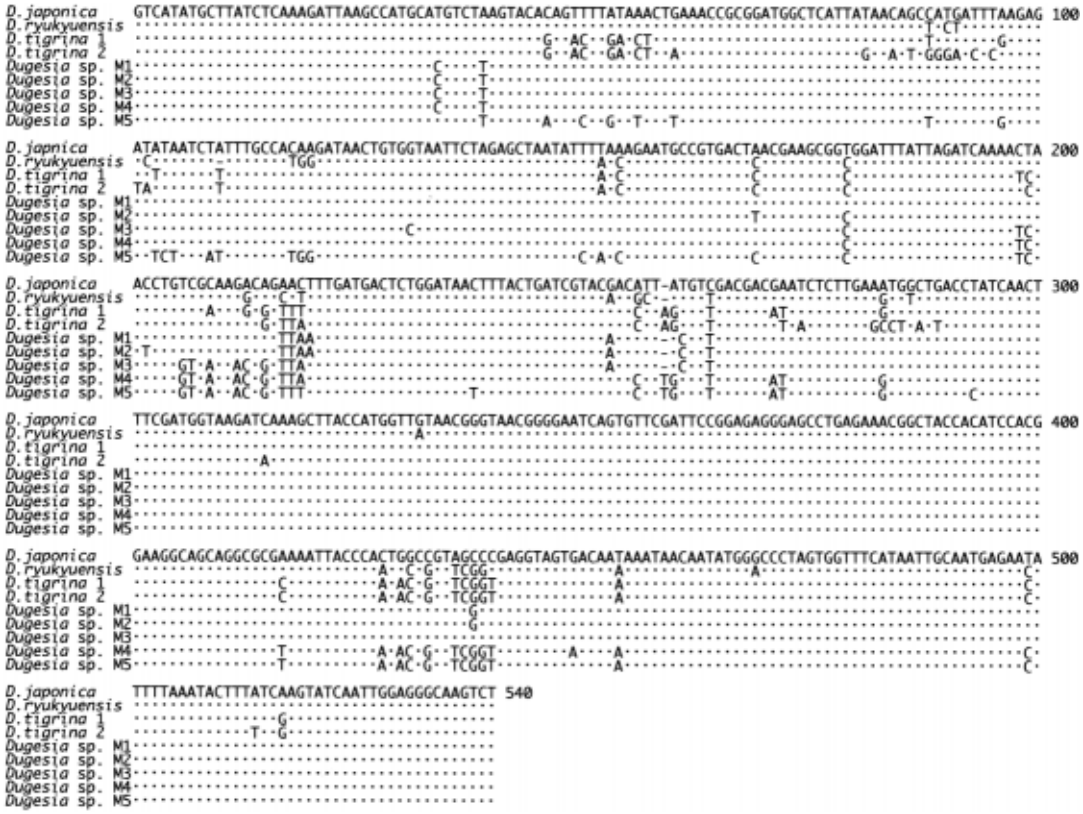

Fig. 1. – Alignment of nine sequences of partial 18S rDNA from Japanese Dugesiidae species. Dots indicate identity with the sequence of *D. japonica*. Dashes indicate deletions.

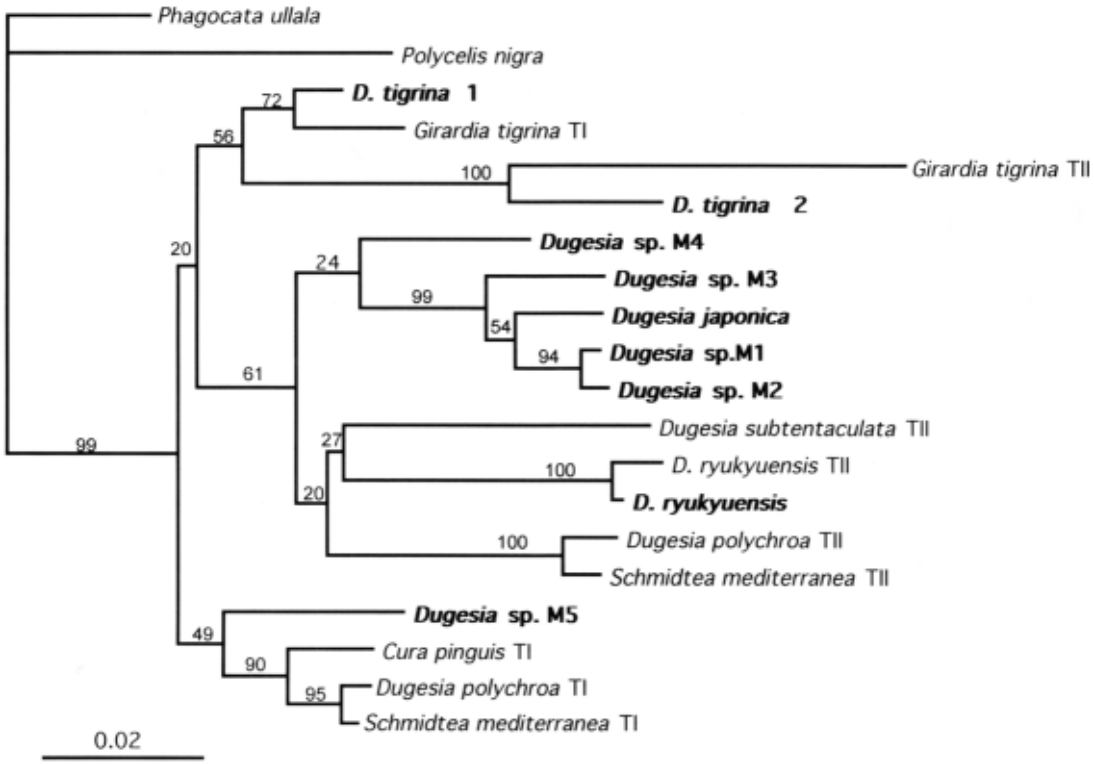

Fig. 2. – The neighbour joining tree for Japanese Dugesiidae and other Dugesiidae from the 18S rDNA sequences. The sequences in bold face were determined by this study and the other sequences have been deposited in GenBank (TI : typeI, TII : typeII). The sequences of *Phagocata ullala* and *Polycelis nigra* were used as outgroups. Bootstrap support (%; n=1000) indicated above the nodes. The scale means that line length equalizes to 0.02 genetic distance calculated by kimura's formula.

northern region of Japan that appears to be geographical intraspecific or interspecific variation.

In the *Dugesia* sp. population of Mie there are probably two types of 18S rDNA and at least two variants, but among *D. japonica* of the northern populations we detected only one type.

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